**Research Projects**

**Breeding Technology**

**Project Title**: Effect of Natural Diets on Ovarian Maturation Stages of Orange Mud Crab, *Scylla olivacea* (HERBST, 1796).

**Project Leader**: Assoc. Prof. Dr. Mhd. Ikhwanuddin @ Polity bin Abdullah

**Researcher**: Azmie Ghazali (Science Officer)

This study was aimed to describe the ovarian maturation stage of wild orange mud Crab, *Scylla olivacea* via morphological observation, histological study and fatty acid composition. Samples were collected from the wild habitat of Kedah Coastal Water, Malaysia from May to July 2011. Ovarian maturation of *S. olivacea* was classified into four stages: Stage 1, Stage 2, Stage 3 and Stage 4. Morphologically, Stage 1 ovary was translucent to off white in color. As the ovary develop the color start to change from pale yellow or light peach (Stage 2) to light yellow or light orange (Stage 3), eventually to reddish orange (Stage 4). Gonadosomatic index (GSI) of wild crabs remained low during Stage 1 and Stage 2 but increased significantly (P<0.05) in Stage 3 and Stage 4 ovaries. Mean GSI at Stage 1 to Stage 4 ovary was 2.46±1.31 %, 3.46±2.29 %, 7.65±3.50 % and 10.71±4.29 % respectively.

Histologically, oocytes size were significantly different (P<0.05) in all ovarian maturation stages of wild crabs. Stage 1 ovary of wild crabs was indicated by the presence of oogonia, follicle cells and primary oocytes. Follicle cells observed surrounds the oocyte. Mean oocyte diameter of Stage 1 ovary of wild crab was 70.89±7.75 µm. During Stage 2 ovary of wild crabs, small yolk globules start to appear in advanced oocytes with follicle cells still observed surrounding the oocytes. The nucleus size is obviously large in oocytes of all crabs. Mean oocytes diameter of Stage 2 ovary of wild crabs was 81.87±6.04 µm. Stage 3 ovary of wild crabs was indicated by the mass size of oocytes and obvious appearance of yolk globules in the cytoplasm of oocytes. Follicle cells were hardly recognized and flattened. Shrinking nucleus indicates the beginning of germinal vesicle breakdown (GVBD). Mean oocytes diameter of Stage 3 ovary of wild crabs was 139.14±14.06 µm. Stage 4 ovary of wild caught crabs could be easily indicated by the appearance of large and fused yolk globules in the oocytes with nucleus was barely visible and large oocytes size with mean oocytes diameter of 177.63±11.35 µm.

Fatty acid composition examination during ovarian maturation of *S. olivacea* indicates that, concentration of total fatty acids, saturated fatty acids (SAFA), monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) in wild crabs was increased from Stage 1 ovary to Stage 3 ovary. Concentration total fatty acids, SAFA, MUFA and PUFA was declined in Stage 4 of all crabs but not significantly differ (P>0.05) from the previous stage. The present study revealed that, ovarian maturation stages of *S. olivacea* are closely related to its morphological appearance (e.g. ovary color and shape), cellular development (e.g. oocyte size and GSI) and biochemical composition (e.g. fatty acids concentration). The ovarian maturation stage classification of *S. olivacea* provides important information for further studies on reproductive biology of this species.
**Morphological and Histological Appearance**

**Figure 1**: Morphological and histological appearance of Stage 1 ovary of wild caught *S. olivacea* with translucent to off white ovary. Histological section of Stage 1 ovary of *S. olivacea* showing oogonia (Og), follicle cells (Fc) and primary oocytes (Po) in each lobe. The follicle cells surround the larger oocytes with oocyte contain a large nucleus (N). O-ovary, H-hepatopancreas, Fg-foregut.

**Figure 2**: Morphological appearance of Stage 2 ovary of wild caught *S. olivacea* with pale to light yellow, and sometimes peach-like ovary. Histological section of Stage 2 ovary of *S. olivacea* showing yolk globule (Yg) start to appear in the cytoplasm of advance oocyte. Follicle cells (Fc) still observed during this stage. Size of nucleus (N) was still large and nucleolus (Nc) obviously observed. O-ovary, H-hepatopancreas, Fg-foregut.

**Figure 3**: Morphological appearance of Stage 3 ovary of wild caught *S. olivacea* with yellow to light orange ovary. Histological section of Stage 3 ovary of *S. olivacea* showing the decreasing size of nucleus (N). Yolk globules (Yg) also increased and filled the cytoplasm of oocyte. O-ovary, H-hepatopancreas, Fg-foregut.
Figure 4: Morphological appearance of Stage 4 ovary of wild caught *S. olivacea* with orange to reddish orange ovary. Histological section of Stage 4 ovary of *S. olivacea* showing larger oocytes with large yolk globules (Yg) fused to each other and occupying the entire cytoplasm. Nucleolus (Nc) is barely visible. There was no record for Stage 4 ovary of scadfish-fed crabs due to no production of Stage 4 ovary after 60 days study period. O-ovary, H-hepatopancreas

Figure 5: Concentration of saturated fatty acids (SAFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) of wild caught crabs at different ovarian maturation.
Project title: Mating Success of Hybrid Trials between Two Mud Crab Species, *Scylla tranquebarica* and *Scylla olivacea*.

Project leader: Assoc. Prof. Dr. Mhd. Ikhwanuddin @ Polity bin Abdullah

Researchers: Noor Baiduri Shaibani
             Nurul Akmal Sudin

This study was done to investigate the mating success between two mud crab species, *S. olivacea* and *S. tranquebarica*, by determining the duration and record the process of mating activity (Fig. 2). Wild mud crab samples were collected from Terengganu coastal water, Malaysia. Limb autotomy was subjected to female crabs to induce molting before the introduction of male crab for mating trials. 10 pairs of each mating trial were observed.

Mating success of control trials of *S. olivacea* is 60% (T1) and *S. tranquebarica* is 50% (T2). Mating success of hybrid trials for male *S. olivacea* with female *S. tranquebarica* is 40% (T3), and male *S. tranquebarica* with female *S. olivacea* is 30% (T4). The highest mean duration of pre-copulatory guarding is T3 (12,240 min ± 1,859.0), followed by T1 (8,840 min ± 43,653.2), T4 (8,666.7 min ± 2,199.6) and T2 (8,064 min ± 1,287.9). As for copulation, the highest mean duration is T4 (59.3 min ± 18.0) followed by T3 (47.5 min ± 6.5), T2 (36.0 min ± 3.8) and T1 (59.3 min ± 18.0). Meanwhile, the highest mean duration of post-copulatory guarding is T2 (312.0 min ± 50.2) followed by T1 (280.0 min ± 49.0), T4 (90.0 min ± 30.0) and T3 (82.5 min ± 28.7). Longer duration of pre-copulatory guarding and copulation were observed on hybrid trials (T3, T4) compare to the controls (T1, T2) (Fig. 1).

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<th>Male (♂)</th>
<th>Female (♀)</th>
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<td>T1</td>
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<td><em>S. olivacea</em></td>
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<td>T2</td>
<td><em>S. tranquebarica</em></td>
<td><em>S. tranquebarica</em></td>
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<td>T3</td>
<td><em>S. olivacea</em></td>
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<td>T4</td>
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Fig. 1. Bar chart shows the (i) percentage of mating success; and mean duration of (ii) pre-copulatory guarding, minute; (iii) copulation, minute; and (iv) post-copulation, minute; for different mating trials.

Fig. 2. The two mud crab species used in the present study, (i) *Scylla olivacea* and (ii) *Scylla tranquebarica*. 
It has been observed that all successful mating trials in the present study show general mating activities, involving pre-copulatory guarding, copulation and post-copulatory guarding (Fig.3). Result of the present study shows that hybridization can occur in captivity and there are also possibilities of hybridization between the two mud crab species to occur in the wild.

Fig.3. The mating activities involving (i) pre-copulatory guarding, (ii) copulation and (iii) post-copulatory guarding of hybrid trials between two mud crab species, *S. olivacea* and *S. tranquebarica.*
Hatchery trials were conducted to establish the effects of a molting hormone, 20-hydroxyecdysone (20E) as water additives on the larval survival, growth and development rate of blue swimming crab, *Portunus pelagicus*. Berried female was brought from Johor coastal water (Fig. 1a, 1b). All experiment were six replicate and designed in three different administration of 20E 0.1 ng mL\(^{-1}\) (Treatment 1-T1), 0.5 ng mL\(^{-1}\) (Treatment 2-T2) and 1.0 ng mL\(^{-1}\) (Treatment 3-T3) were added daily and one control without any 20E.

Day one after hatch (DAH) larvae (Fig. 2) at Zoea 1 (Fig. 3) was used for this experiment until it reach the megalopa stage (Fig. 4). The addition of 20E significantly increased survival rate (p<0.05) for all treatments over controls. Highest survival rate (13.0±0.5%) of larvae was achieved in T2. The results show that 20E plays a significant role during larval development of crabs. The results of present study suggest that the molting hormone, 20E added to rearing water at a certain concentration significantly improved survival rate and enhanced larval development of *P. pelagicus* larvae. Based on the result of the current study of molting hormone, 20E are recommended for mass seed production practices. They might synchronize larval development and accelerating larval molting of crustacean.
One of the most critical point for producing gynogenetic diploid was studied in banana shrimp, *Fenneropenaeus merguiensis* (Fig. 1). A fully matured male broodstock was taken with fresh spermatophores and the sperm chromosomes were inactivated with UV irradiation for 20-80 sec at 254nm and 365nm. Sperms counts were determined using modified eosin-nigrosin staining method. Percentage of sperm viability was decreasing when duration of exposure increasing. Sperm exposed to 20 sec of UV irradiation with 254nm produce mean 90.68% of viability, however, 80 sec of UV irradiation with 365nm produce mean 16.64% of viability (Fig. 2). The irradiated sperms and fresh sperms show a similarity in term of viability. The viability was high and observation under Scanning Electron Microscope (SEM) and Tabletop Microscope shows there was no physical changes or damage occurred on the sperm structure (Figure 3).

Fig. 1: Banana shrimp, *Fenneropenaeus merguiensis*.  

![Banana shrimp](image1)

Fig. 2: Mean viability percentages of sperms with different UV duration and wavelength. Different latter indicate significant difference among treatments (p<0.05). The mean viability percentages for each treatments was; 20s 254nm = 90.68; 20s 65nm = 82.71; 40s 254nm = 78.45; 40s 365nm = 74.95; 60s 254nm = 75.35; 60s 365nm = 74.46; 80s 254nm = 49.7; and 80s 365nm = 16.67.
Project title: The effects of triploidy towards growth performance, survival rate, gender ratio and sex differentiation on Banana Shrimp, *Penaeus merguiensis* (de Man, 1888) postlarvae compared to diploid siblings.

Project leader: Assoc. Prof. Dr. Mhd. Ikhwanuddin @ Polity bin Abdullah

Researchers: Assoc. Prof. Dr. Abol Munafi Ambok Bolong
Dr. Shahreza Sheriff
Norhidayah Binti Abdul Manan (M.Sc. Student)

Study was done to show the effect of triploidy on growth performance, survival rate and gender ratio on Banana shrimp, *Penaeus merguiensis* compared to diploid sibling otherwise identifying the sex external characteristic of the postlarval. Cold shocks (10°C, 15°C and 20°C) with control (28°C) and three different time of exposures (10, 15 and 20 min) were introduced to each treatments. The fertilization rate was significantly different (*P* < 0.05) among treatments and the hatching rate was identified no significantly different (*P* > 0.05). Larvae got poor survival rate when reached mysis stage and there was no significantly different (*P* > 0.05) among each treatments. Triploidy were identified in 15°C treatment for all time of exposures and other treatments produced diploid. 15°C treatment and control were reared until postlarval 50 and the survival rate were significantly different (*P* < 0.05). Male shrimps were differentiated by present of petasma (fig. 1. a, b, c) and male gonophores (mg), (fig. 2. a, b) and females were differentiated by appearance of sharp ridges thelycum (srt), (fig 3. a, b) . For the gender ratio, it was significantly higher differences (*P* < 0.05) where 15°C treatment produced 94% males compared to control that produced 85% females. For growth performance of total length and body weight, there were significantly different between control and triploid treatments, even though have a quite similar proportional for each treatments. The Specific growth rate (SGR) obtained were also identified significantly different (*P* < 0.05) between control and the 15°C treatment.
Fig. 1: Males were differentiated from female by the appeared of Petasma (P) on the first swimming leg of Postlarvae 50 shrimp using dissecting microscopes (a) and advance microscopes for (b) and advance microscope for (c) (10x).

Fig. 2: (a) Control males; (b) Treated males with the appearance of petasma (P) at the first swimming leg and male gonophores (mg) at the fifth walking legs using dissecting microscope (10x).

Fig. 3: (a) Control females; (b) Treated females were differentiated with male by the appeared of sharp ridges thelycum (Srt) using dissecting microscopes (10x).
The objective of this study was to determine the effect of temperature on the embryonic development of Mud Spiny Lobster, *Panulirus polyphagus*. 5 berried females were used and kept at different temperature 25-28°C (control), 20°C (low temperature) and 30°C (high temperature). In control, the largest and smallest mean diameters was 0.668 mm ± 0.037 (Day 11) and 0.520 mm ± 0.023 (Day 1). In low temperature (Replicate 1 and 2), the largest mean diameter was 0.632 mm ± 0.039 (Day 5) and 0.598 mm ± 0.041 (Day 17) and smallest mean diameter was 0.535 mm ± 0.042 and 0.520 mm ± 0.028 (Day 3), respectively. In high temperature (Replicate 1 and 2), the largest mean diameter was 0.651 mm ± 0.012 (Day 4) and 0.679 mm ± 0.022 (Day 3), respectively. At the same time, the smallest mean diameter was 0.522 mm ± 0.018 (Day 2) and 0.567 mm ± 0.042 (Day 1), respectively. In conclusion, incubation of berried female in low temperature could cause mortality to the lobster and trigger the berried female to release the eggs unhatc due to stress.

Color change phenomenon during eggs maturation period; (A) Early stage - Bright orange; (B) Middle stage - Brick red; (C) Prior to hatching - Dark red.
Figure 1: Embryonic development of *P. polyphagus* berried female stages; **ME;** Median Eye, **Y;** Egg Yolk, **f;** funiculus, **E;** Eyespot, **C;** Chromatophore. (Magnification: 10 ×).
Project title: The effect of fatty acids from crickets, *Gryllus* sp. on reproductive performance of broodstock females of *Macrobrachium rosenbergii*.

Project leader: Dr Safiah Jasmani

Researchers: Jannatul Farihah binti Zulkifli (M.Sc. Student)

Reproductive performance of female *Macrobrachium rosenbergii* broodstock is very important to give high seed production. Fatty acids composition of shrimp eggs has been strongly indicated affected by the source of lipid in the broodstock diet. Some essential fatty acids (EFA) have also been shown to be of special significance for gonad maturation and brood quality. High level of polyunsaturated fatty acids has been shown improved fecundity, egg hatchability, and the overall quality of *M. rosenbergii* larvae.

As one of natural food sources, cricket, *Gryllus* sp. contains a lot of nutrients that is good for human consumption. Many previous studies have been carried out on the total lipid composition of the black cricket, phospholipid and triglyceride fatty acid compositions of *Melanogryllus desertus* and the distribution in lipid classes of fatty acids biosynthesized by the black cricket. Their fatty acids pattern resembled those of some fish oils, and concentration of total PUFA accounted for 46% of total fatty acids. These characteristics make them the best candidate as the fatty acids source for ovarian maturation in shrimp.

In *M. rosenbergii*, there are molting and reproductive cycles which closely linked under the control of X-organ/sinus gland complex. When maintained in captivity about 60% of the broodstock females spawned eggs after reproductive molt, whereas 40% undergone non-reproductive molt. Although the regulation mechanism is not clear it is presumed that when somatic growth dominates intermittent non-reproductive molts take place. In hatchery operation this is a limiting factor to maximize production and fulfill market demand.

Thus, the information from this study will contribute to better understanding of fatty acids role in ovarian development, rematuration and continuous breeding of *M. rosenbergii*. Moreover, this data will give information about natural food like cricket in stimulating gonad maturation of crustacean. This information can be used to help the farmers in Malaysia to stock good quality of broodstocks and produce the high yield with low cost budget to fulfill the demand.
Aquaculture Engineering

Project title: Growth performances and survival rates of post larval of *Macrobrachium rosenbergii* (de Mann) grown at varying stocking densities during nursery phase using Greenwater, Clearwater and Recirculating Water System (RAS).

Project leader: Assoc. Prof. Aizam Zainal Abidin.

Researchers: Mohd Asri bin Zulkifli (M.Sc. Student)

The Giant freshwater prawn, *Macrobrachium rosenbergii* known as Malaysian Freshwater Giant Prawn is one of the most preferred species for culture and now getting more attention from the aquaculturist worldwide due to its high market demand. The nursery systems were defined as an intermediate phase between larval rearing stage and grow-out pond. This phase is very important especially to culturists because the climatic on survival of prawn and water restriction cannot allow continuous culture (New 1990, 1995). The objective of this study is to compare the growth performances and survival rates of post larval (PL), *Macrobrachium rosenbergii* grown at varying stocking densities during nursery phase in different culture systems in tanks of size 175cm x 95cm x 70cm in 200 liters of water.

Result of cycle 1: Stocking rate of 600PL/200 liter of water.

![Growth performance (weight) of post larval *Macrobrachium rosenbergii*](image1.png)

**Figure 1:** Comparison of the growth performance (body of weight) in different culture system at stocking rate of 3PL/Liter of water.

![Growth performance (body of length) of post larval *Macrobrachium rosenbergii*](image2.png)

**Figure 2:** Comparison of the growth rates (body of length) in different culture system at stocking rate of 3PL/Liter of water.
Figure 3: Comparison of the survival rates in clearwater, greenwater and recirculating system (RAS) stocking rate of 3PL/Liter of water.