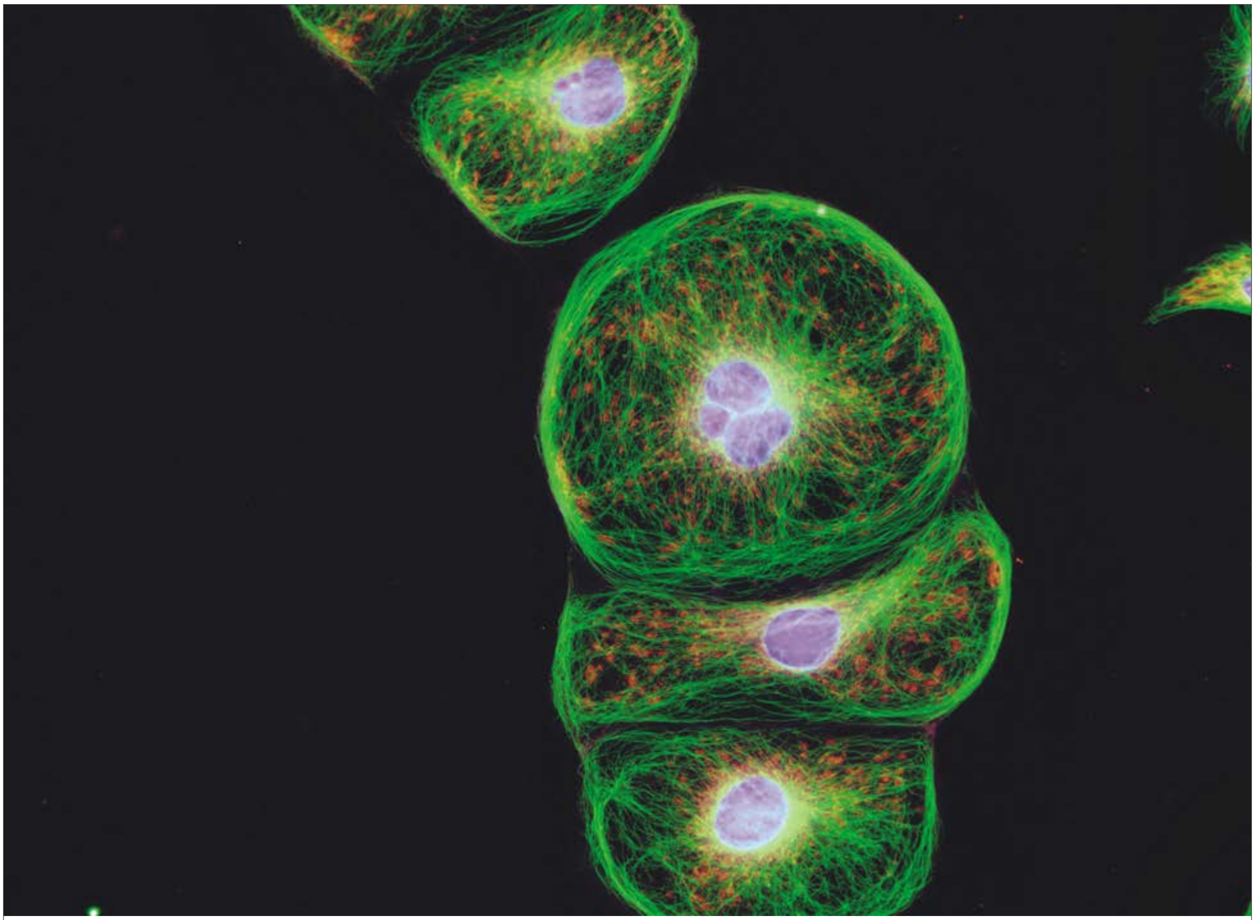

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Reproductive Biology on the Gonad of Female Orange Mud Crab, *Scylla olivacea* (Herbst, 1796) from the West Coastal Water of Peninsular Malaysia

[1]M. Ikhwanuddin, [2]J. Nur-Atika, [2]A.B. Abol-Munafi and [1]H. Muhd-Farouk
[1]Institute of Tropical Aquaculture,
[2]School of Fisheries and Aquaculture Sciences, Universiti Malaysia Terengganu, Kuala Terengganu, 21030, Terengganu, Malaysia

Corresponding Author: M. Ikhwanuddin, Institute of Tropical Aquaculture, Universiti Malaysia Terengganu, Kuala Terengganu, 21030, Terengganu, Malaysia

ABSTRACT

This study describes the female size at sexual maturity (CW_{50}) and the ovarian maturation stages of orange mud crab, *S. olivacea* through ovary external morphological and histological descriptions from west coast of peninsular Malaysia. All female *S. olivacea* samples were sampled from Kedah coastal waters of west coast of Peninsular Malaysia. The Carapace Width (CW) and CW_{50} was taken for external morphological descriptions and the ovary were used in the histological descriptions to observe the size of their oocyte based on each ovarian maturation stages. From this study, *S. olivacea* female CW_{50} is 8.9 cm. The ovary colour changes in every ovarian maturation stages ranging from translucent for stage 1, yellowish during stage 2, orange in stage 3 and finally the ovary turned to dark orange during stage 4 when the ovary are well developed with the mean oocytes diameter of 28.29±7.79 µm for stage 1, 68.1±13.68 µm for stage 2, 80.97±13.69 µm for stage 3 and 130±21.08 µm for stage 4. Likewise the size of the oocytes in the ovaries increases as the ovary developed further from ovarian maturation stage 1-4.

Key words: Sexual maturity, ovarian maturation stages, carapace width, orange mud crab, *Scylla olivacea*

INTRODUCTION

Mud crab, genus *Scylla* is a commercially important portunid crab and it is traditionally exploited by artisanal fishermen and provides a basic source of income for coastal fishing communities throughout the Indo-Pacific region (Islam *et al.*, 2010). Productions of mud crab through aquaculture in some country are becoming important as an export commodity. Mud crab had become one of the important export commodity that leads to many big opportunities in crab farming (Begum *et al.*, 2009). Southeast Asia had become one of the earliest to practice mud crab aquaculture which mostly focused on capture and fattening of the juvenile crabs from wild (Begum *et al.*, 2009) as they are highly demanded for all size classes, including mature females for a premium market and soft-shell mud crab culture for new food materials (Marichamy and Rajapackam, 2001). Since the first commercial mud crab aquaculture operation began in Malaysia in 1991, the mud crab has gradually entered local markets and become a main component of the local crab fishery (Ikhwanuddin *et al.*, 2011). Demand for this species had been increasing because of the qualities it possesses, including large size, high meat yield (Rattanachote and Dangwatanakul, 1992) and rapid growth during culture (Millamena and Quinitio, 1999). Few studies had been done so far on reproductive biology of the orange mud crab, *Scylla olivacea* in Malaysia (Ikhwanuddin *et al.*, 2011). This reproductive biology information gathers from this study on sexual maturity and gonad maturation are important to understand the behavior and characteristic of *S. olivacea* for the management of mud crab resources exploitation for capture fishery and aquaculture purpose. The objectives of this study are to determine the female size at sexual maturity and to record the ovarian maturation stages of orange mud crab, *S. olivacea* through ovary external morphological and histological descriptions from west coast of Peninsular Malaysia.

MATERIALS AND METHODS

Crab samples: All female *S. olivacea* samples were sampled from Kedah coastal waters of west coast of Peninsular Malaysia (Lat. 5° 34' 58 N; Long. 100° 22' 58 E). A total of 40 female crabs were randomly collected from various sizes and dissected to determine the carapace width at 50% sexual maturity (CW_{50}). For each ovarian maturation stages of stage 1-4, ovary from 10 different crab individuals were processed for the external and histological assessments.

Crab morphometrics: The size of crabs was measured as the Carapace Width (CW) which is the distance, between the tips of 9th antero-lateral spines of the carapace. The measurement of the CW was measures by using the vernier caliper where it was measured to the nearest 0.1 cm. The Body Weight (BW) of crabs was measured individually to the nearest 0.1 g using digital electronic balance.

Size at sexual maturity: All female crab's abdominal flaps were observed to determine the preliminary stage of maturity. Matured female crabs have a large round pigmented abdominal flap while the immature female crabs have small and pale abdominal flap (Ikhwanuddin *et al.*, 2010) (Fig. 1).

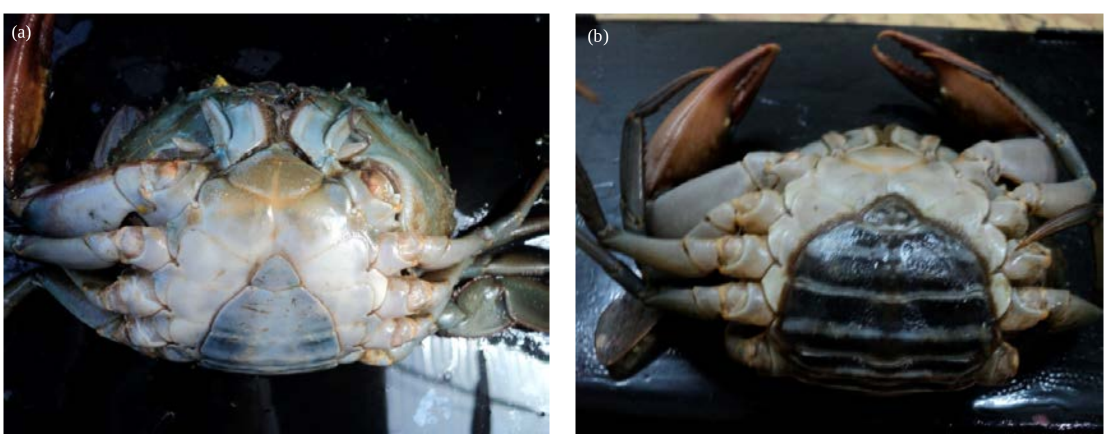

Fig. 1(a-b): (a) Immature female with small and pale abdominal and (b) Mature female with large round pigmented abdominal flap of *Scylla olivacea*

Then, the female crabs were dissected for determining the CW_{50} based on ovarian maturation stages. The ovarian maturation stages were determined from the ovary examination of the external morphological and histological appearances (Azmie *et al*., 2012).

Ovarian maturation stages assessment: Crabs were dissected and carapaces of the crabs were removed in order to determine the external morphology of the ovary. A small portion of ovarian lobes were fixed in Bouin's solution between 12-24 h. The ratio of the tissue block compare to volume of fixative was 1:10. Then, the ovary was removed into the 70% alcohol solution before proceed with tissue processing. Tissue processing was done in the Automatic Tissue Processor for 17 h at 60°C to infiltrate the fixed tissue sample. After processing and hydration of tissue, wax impregnation was done through embedding processed in a paraffin wax to form a solid block. This process was purposely done to provide an easy way to handle solid block when sectioning with microtome. After that, the solid block was cut into 5 µm sections by using a rotary microtome (Leica RM2135). Then, the sections were placed on the paraffin section water bath with temperature maintained at 40-45°C and allowed to expand. Once the sections expand in full size, a microscope slide was held at an angle and slid under one or two well-formed tissue sections. After the tissues were dried on hot plate (60°C) for overnight, the samples were continued with staining procedure of modified eosin-haematoxylin stain by staining in haematoxylin solution, decolorizing in 1% acid alcohol dips, contrasted in eosin solution 0.5% aqueous, dehydrated in a series of ethanol concentrations, cleared in xylenes and mounted in synthetic resin mounting medium DPX. The diameter of oocytes for each ovarian maturation stages was measured under an advanced research microscope (Leica Microsystem, Wetzlar GmbH, DM LB 2, Germany).

Data analysis: The size when 50% of the crab was sexually matured was chosen as appropriate measure of the size at sexual maturity (CW_{50}) in female crabs. The CW_{50} was determined by using the sigmoid curve at any CW ranges that shows percentage of maturity. The diameter of 30 oocytes for each ovarian maturation stages was presented as means and standard deviation through Microsoft Office Excel 2007.

RESULTS

Size at sexual maturity: The 10 out of 40 measured female *S. olivacea* had immature ovary which is stage 1. The largest immature female encountered was at 10.5 cm CW and the smallest mature female was at 8.18 cm CW. The mean value of size of CW_{50} of female crab was at 10.44±1.18 cm (Table 1). The CW range frequency of females sampled was most frequently found

Table 1: Mean of female *Scylla olivacea* size (cm) for mature and immature ovary based on ovarian maturation stages

Parameters	Mature ovary	Immature ovary
Mean	10.44	8.19
Max	12.90	10.50
Min	8.18	6.28
SD	1.18	1.42
N	30.00	10.00
Total sample	40.00	

Mature ovary: Ovary within the ovarian maturation stages 2, 3, 4, Immature ovary: Ovary of ovarian maturation stage 1 in size range of 10.0-10.5 cm CW (20.0%) (Table 2). Percentage of mature female was calculated for each 0.5 cm CW interval from CW range of 6.0 cm (the smallest size range of the crab sampled) to CW range of 13.0 cm (the largest size range of the crab sampled). Using the sigmoid curve of the CW ranges that shows percentage of maturity, CW_{50} occurred at 8.09 cm CW in the female crabs (Fig. 2).

Ovarian maturation stages: The progress of ovarian maturation was classified into four stages based on external morphological characteristic (Table 3) and histological characteristic (Fig. 3 and Table 3).

Table 2: Carapace width (cm) frequency, percentage frequency, maturity frequency and maturity percentage of mature female *Scylla olivacea* based on ovarian maturation stages

Size range (cm)	Frequency	Percentage	Maturity frequency	Maturity (%)
6.0-6.5	1	2.5	0	0
6.5-7.0	2	5.0	0	0
7.0-7.5	1	2.5	0	0
7.5-8.0	0	0.0	0	0
8.0-8.5	3	7.5	1	33.3
8.5-9.0	3	7.5	2	66.6
9.0-9.5	4	10.0	3	75
9.5-10.0	7	17.5	6	85.7
10.0-10.5	8	20.0	7	87.5
10.5-11.0	3	7.5	3	100
11.0-11.5	3	7.5	3	100
11.5-12.0	1	2.5	1	100
12.0-12.5	1	2.5	1	100
12.5-13.0	3	7.5	3	100
Total	40	10.0	0	30

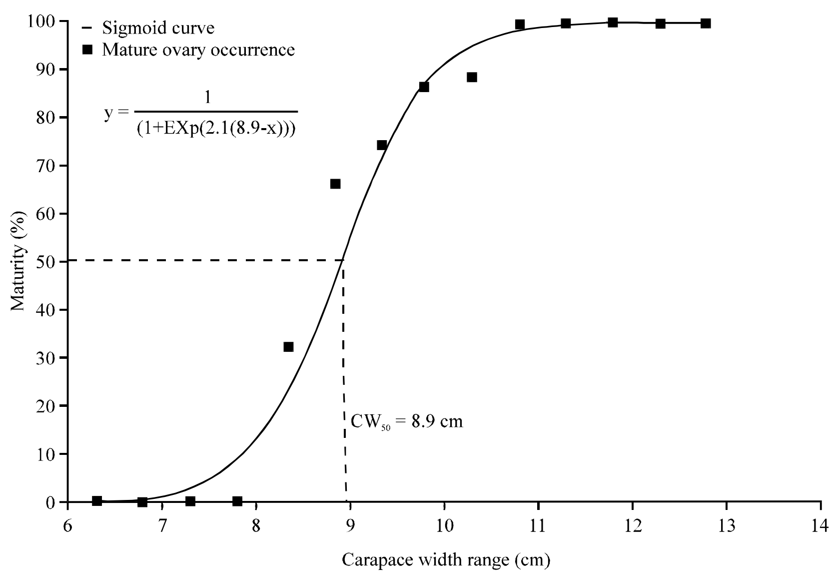

Fig. 2: Size at maturity (CW_{50}) from sigmoid curve of the carapace with ranges that shows percentage of maturity of *S. olivacea* female, where CW_{50} = 8.9 cm

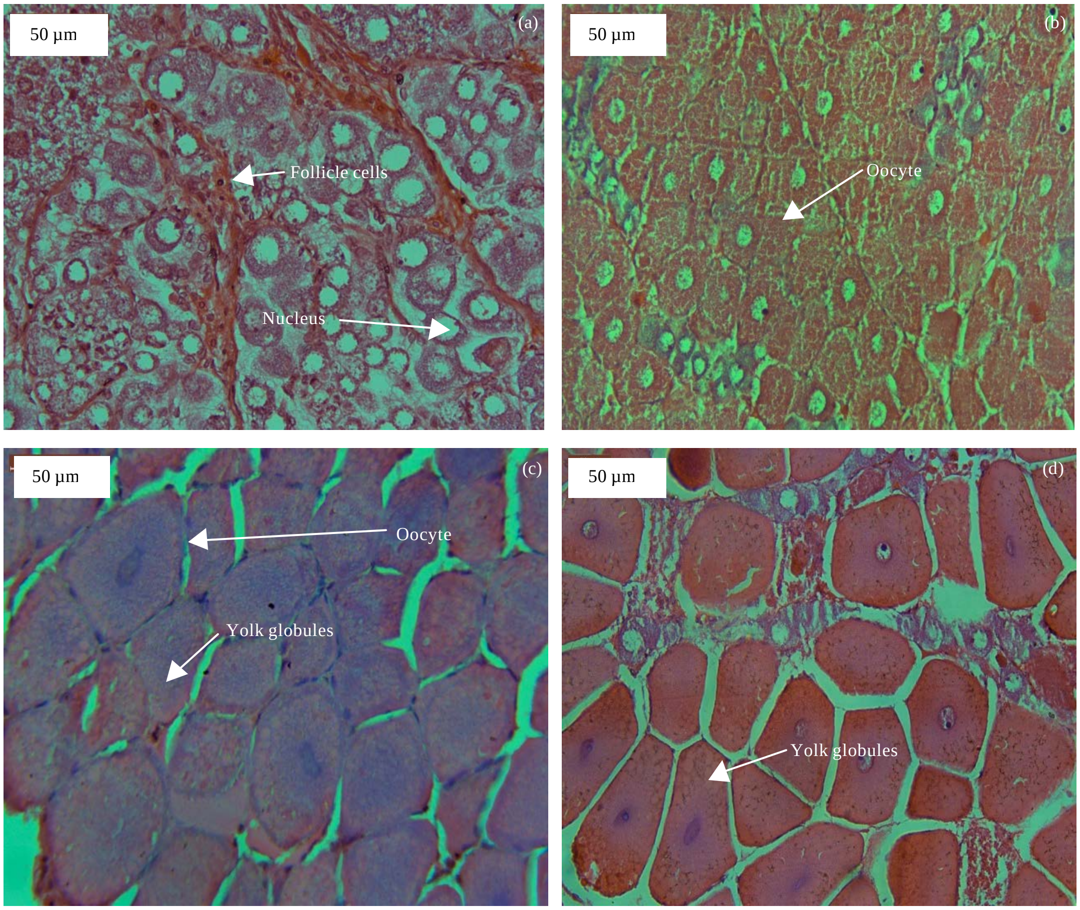

Fig. 3(a-d): Four ovarian developmental stages of *Scylla olivacea* based on the histological assessment, (a) Immature (Stage 1), (b) Early maturing (Stage 2), (c) Pre-maturing (Stage 3) and (d) Fully matured (Stage 4)

Table 3: External morphological and histological characteristics of the ovarian maturation stages of *Scylla olivacea*

Ovarian maturation stages	External morphological characteristics	Histological characteristics
Immature (Stage 1)	Translucent jelly like to pale white ovary with ovary showing ribbon-like structure	Mean oocyte diameter 28.29±7.79 µm. Presence of cluster of oogonia, oocytes and follicle cells in the ovarian lobe. Follicle cells found around the periphery of the lobes and in an area among groups of oogonia and oocytes
Early maturing (Stage 2)	Ovary pale or light yellow in colour and increases in size	Mean oocyte diameter 68.0±13.68 µm. Small yolk globules start to appear in larger oocytes, follicle cells around the oocytes
Pre-maturing (Stage 3)	Increase in ovarian size, yellow to orange ovary	Mean oocyte diameter 80.97±13.69 µm. Yolk globules occur in the cytoplasm with larger globular, follicle cells hardly visible
Fully matured (Stage 4)	Ovary occupies available space in body cavity; orange to red orange ovary	Mean oocyte diameter 130.43±21.08 µm. Large yolk globules in the entire cytoplasm, nucleus small, follicle cells hardly seen

Fig. 4(a-d): External morphological characteristic of *Scylla olivacea* ovarian maturation stages, (a) Immature (Stage 1), (b) Early maturing (Stage 2), (c) Pre-maturing (Stage 3) and (d) Fully matured (Stage 4)

The observation of the ovaries was determined by four stages (Fig. 3 and 4). Immature ovary (stage 1) was observed as a strand of transparent to translucent tissue that was sometimes difficult to recognize. The mean oocyte diameter recorded in stage 1 was the lowest (28.29±7.79 µm) as the oocytes were developing towards the mature oocytes. The follicle cells were small and distributed around the periphery of the lobes and in an area among groups of oogonia and oocytes. The follicle cells gradually enclosed each oocyte. Early maturing ovary (stage 2) was yellow and small yolk globules started to appear inside the larger oocytes. Mean oocyte diameter (68.1±13.68 µm) was increased indicating the progression in development of oocytes size. Late-maturing ovary (stage 3) was light orange and lobules were apparent. Yolk globules occurred in the cytoplasm with larger globular inclusion towards the periphery while follicle cells were hardly visible. In this stage, the oocyte grows rapidly as the mean oocyte diameters (Table 4) is 80.97±13.69 µm increased and the nucleus also reached maximum size. When fully matured (stage 4), the ovary was orange to deep orange. The ovary enlarged and eventually covered the hepatopancreas and the majority of the cardiac stomach. Large yolk globules were apparent in the entire cytoplasm and partly fused to one another (Table 3). There was a conspicuous increase in the oocyte diameter 130±21.08 µm (Table 4).

Table 4: Mean Body Weight (BW), Carapace Width (CW) and oocyte diameter based on the different ovarian maturation stages of *Scylla olivacea*

Ovarian maturation stages	BW (g)	CW (cm)	Oocyte diameter (μm)
Immature (Stage 1)	98.60±44.07	8.11±1.46	28.29±7.79
Early maturing (Stage 2)	163.39±40.02	9.45±0.82	68.10±13.68
Pre-maturing (Stage 3)	164.93±32.09	10.14±0.60	80.97±13.69
Fully matured (Stage 4)	174.65±48.39	11.66±0.91	130.43±21.08

DISCUSSION

Sexual maturity is defined as the ability to mate and extrude of fertilised eggs (Ikhwanuddin *et al.*, 2011). The pubertal moult stage in *Scylla* spp. is said to be a reliable indicator of the ability of female crab to mate (Ikhwanuddin *et al.*, 2011). Studies have also shown that the female of *Scylla* spp. will moult again after the pubertal moult (Heasman, 1980; Ong, 1966; Quinn and Kojis, 1987; Robertson and Kruger, 1994). The size at sexual maturity of female *S. olivacea* from the present study (CW_{50} = 9.08 cm CW) is almost similar with the result for female *S. olivacea*, 9.06 cm CW from Terengganu coastal waters, east coast of Peninsular Malaysia (Ikhwanuddin *et al.*, 2010). Moreover, Ikhwanuddin *et al.* (2011) mentioned the CW_{50} female of *S. olivacea* from mangrove areas of Sarawak coastal waters, East Malaysia at 8.6 cm CW that is smaller than the result of present study. The results of present study support the report by Ikhwanuddin *et al.* (2010) where the size of maturity is related to the geographical differences. It has been reported that the size at sexual maturity is attained by brachyuran crabs may vary from place to place (Rasheed and Mustaquim, 2010). Hines (1989) compared geographic variation in size of sexually mature females in five species of crabs along the east and west coast of North America (Rasheed and Mustaquim, 2010). Four of the five species show significant geographic variation in size at onset of maturity (Rasheed and Mustaquim, 2010). The differences in size at sexual maturity among population of the same species of crab also may be attributed to variation in moult increment and in the number of moults (Rasheed and Mustaquim, 2010). Ecological conditions also have an influence on the sexual maturity of mud crab through the amount of available food (Jirapunpipat, 2008) and environmental factors such as temperature and salinity (Rasheed and Mustaquim, 2010). Study by Fisher (1999), also reported that temperature and salinity affected CW_{50} of female blue crab, *Callinectes sapidus* from nine Texas coast, as temperature and salinity vary from bay to bay.

Present study shows that, the diameter of the oocyte (Table 4) increased as the ovarian maturation stages progressed, resulting in an increase in the volume of the ovary as shown by the external observation of the ovary. The major phases of oogenesis in crabs are the previtellogenic phase, which contains essentially primary oocytes and the vitellogenic phase, in which oocytes increase in size as yolk is incorporated into the cytoplasm (Islam *et al.*, 2010). The developing oocytes and the follicle cells are the major cell types within the ovarian lobes of *Scylla* species. Follicle cells in the present study are larger and more obvious in immature gonad, which is similar to those observed in *S. olivacea* by Islam *et al.* (2010). The follicle cells surround each oocytes were flattened as progression of further maturation. These follicle cells might be related to ovarian yolk synthesis in *S. olivacea* as reported in *S. paramamosain* (Islam *et al.*, 2010). According to Ryan (1967), the follicle cells will form the chorionic membrane of the mature ovum.

It was observed in this present study that the colour of the immature ovaries is translucent to yellow and become darker yellow to dark orange in mature ovaries as similar both result obtained by Quinitio *et al.* (2007) for *S. serrata* and for *S. olivacea* but differ with

S. paramamosain (Islam *et al.*, 2010). The phenomenon of changing colouration in ovaries due to accumulation of yolk in the oocytes which serves as the source of nutrition for the developing embryo because of the yolk contains protein, lipids, sugars and some steroid hormones, This variability in colour also may be due to the diet intake of the crab (Quinitio *et al.*, 2007). There was difference between mean oocyte diameters from this study and previous study by Islam *et al.* (2010) on the similar mud crab species of *S. olivacea* where the difference might be due to the diet intake of the crab and environmental factors. The result of the present study suggests that ovarian maturation stages cannot be determined precisely by morphological observation of the abdomen. However, the initiation of vitellogenesis could be identified by external morphological observation of the ovary without examining ovarian maturation stage histologically.

CONCLUSION

The size at sexual maturity for (CW_{50}) *S. olivacea* from west coast of peninsular Malaysiais 8.9 cm CW for female. Ovarian maturation was classified into four ovarian maturation stages which are immature (stage 1), early maturing (stage 2), pre-maturing (stage 3) and fully matured (stage 4). The colour of gonad changes in every stage ranging from translucent for stage 1, yellowish during stage 2, orange in stage 3 and finally the ovary turned to dark orange during stage 4 when the ovary are well developed with mean diameter oocytes of 28.29±7.79 µm for stage 1,68.1±13.68 µm for stage 2, 80.97±13.69 µm for stage 3 and 130±21.08 µm for stage 4, respectively. Likewise, the size of the oocytes in the ovaries increases with the increasing of maturation stage.

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