

## Ovarian Maturation Stages of Wild and Captive Mud Crab, *Scylla olivacea* Fed with Two Diets

(Peringkat Kematangan Ovari bagi Ketam Lumpur Liar dan Kurungan  
*Scylla olivacea* Diberi Makan dengan Dua Diet)

AZMIE GHAZALI, NOORDIYANA MAT NOORDIN, AMBOK BOLONG ABOL-MUNAFI,  
MOHAMAD N. AZRA & MHD IKHWANUDDIN\*

### ABSTRACT

*This study was aimed to determine the ovarian maturation stages of wild and captive orange mud crab, Scylla olivacea fed with different diets via gonadosomatic status, oocyte diameter and histological examinations. Captive crabs were fed with blood cockle, Anadara granosa, or fish, Decapтерus spp. Through the histological examinations, ovarian maturation stages of wild and captive S. olivacea was classified into four stages: Immature (Stage 1), Early maturing (Stage 2), Pre-maturing (Stage 3) and Fully matured (Stage 4). Gonadosomatic Index of wild and captive crabs remained low during immature and 2 but increased significantly ( $p < 0.05$ ) in pre-maturing and 4 ovaries. Oocytes size were significantly different ( $p < 0.05$ ) in all ovarian maturation stages of wild and captive crabs. Follicle cells surround the oocyte of immature ovary and small yolk globules start to appear in early maturing ovary with large nucleus size. Oocyte size increased significantly ( $p < 0.05$ ) and yolk globule obviously appeared in pre-maturing ovary. Large and fused yolk globules appeared in the oocytes of fully matured ovary with nucleus was barely visible. The present study revealed that, ovarian maturation stages of S. olivacea are closely related to its morphological appearance and cellular development.*

*Keywords: Aquatic science; fisheries; invertebrate; nutrition; reproductive biology*

### ABSTRAK

*Kajian ini bertujuan untuk menentukan tahap pematangan ovari bagi ketam lumpur liar dan kurungan jenis Scylla olivacea yang diberi makan dua diet yang berlainan dengan melalui status gonadosomatik, diameter oosit dan pemeriksaan histologi. Ketam lumpur telah diberi makanan seperti kerang darah seperti Anadara granosa, atau ikan seperti Decapтерus spp. Peringkat pematangan ovari bagi S. olivacea liar dan kurungan telah dibahagi kepada empat peringkat melalui pemeriksaan histologi: Tidak matang (Tahap 1), Matang awal (Tahap 2), Pra-matang (Tahap 3) dan Matang sepenuhnya (Tahap 4). Indeks Gonadosomatik ketam lumpur liar dan kurungan adalah rendah pada tahap tidak matang dan 2 ovari tetapi meningkat dengan ketara ( $p < 0.05$ ) bagi pra-matang dan 4 ovari. Saiz oosit adalah berbeza dengan ketara ( $p < 0.05$ ) bagi semua peringkat pematangan ovari bagi ketam lumpur liar dan kurungan. Sel-sel folikel mengelilingi oosit ovuri yang tidak matang dan kuning telur yang kecil mula berlaku pada tahap matang awal ovari dengan saiz nukleus besar. Saiz oosit meningkat secara ketara ( $p < 0.05$ ) dan globul telur kuning jelas kelihatan pada tahap pra-matang ovari.*

*Kata kunci: Biologi pembakan; invertebrat; nutrisi; perikanan; sains akuatik*

### INTRODUCTION

The optimum reproductive performance can only be achieved when the broodstock are supplied with the highly nutritional diets, whether natural diets, formulated diets or mixed of them (Azra & Ikhwanuddin 2016). Obviously, the production of home-made formulated diet in mud crabs relatively consumes high cost time and labour. A variety of highly nutritional diets such as fishes, squids, mollusks, polychaetes have been used to feed the brachyuran broodstock in order to optimize the reproductive performance (Alava et al. 2007; Ikhwanuddin et al. 2015). Although most of the study showed positive findings, the nutritional requirements, however, are species-specific. The need of this information on mud crab is fundamentally important for their sustainable aquaculture production

and management. The quality of food items given to the broodstock are normally reflected by their reproductive performance, especially during ovarian maturation of organism of the cultured species, including mud crab, genus *Scylla* (Azra & Ikhwanuddin 2016).

The mud crab, especially genus *Scylla* formed a better candidate for aquaculture for its faster growth, larger size, high fecundity as well as resistance to disease and adaptability to various farming systems (Viswanathan & Raffi 2015). The status of ovarian maturation in mud crab, especially genus *Scylla* was different based on their locality, experimental treatment and species of crab (Iromo et al. 2015; Islam et al. 2010a; Tantikitti et al. 2015). Classification of ovarian stages of mud crab using morphological characteristics sometimes can vary

from one study to another. Portunid crab normally had an aggressive behavior which is very hard to handle. This factor gives rise to difficulties in the determination of the reproductive biological information of this spiny species (Ikhwanuddin et al. 2011). This is where the relationship between cellular development (histological examination) and reproductive performance (ovarian maturation) is useful. Thus, ovarian maturation stages of *Scylla olivacea* (Herbst 1796) need to be determined and relate its histological appearance of the ovary to oocyte development. By determining this relationship, the use of mature broodstock in the seed production program can be optimized. The reviewed paper by Azra and Ikhwanuddin (2016) indicated that fish and blood cockle usually used as a fed to the mud crab broodstock, especially genus *Scylla*. The main objective of this study was to compare the effects of two different natural diets on ovarian maturation stages of *S. olivacea* via gonadosomatic status, oocyte diameter and histological examinations.

#### MATERIALS AND METHODS

**Crabs samples** All crabs used in the study were sampled from May to July 2014 from Kedah and Terengganu coastal water, Peninsular Malaysia, Malaysia. A total of 240 females of *S. olivacea* of body size between 74.00-86.00 mm carapace widths were sampled at the same time. *S. olivacea* was identified based on the description by Keenan et al. (1998). The crab samples were transferred back to the marine hatchery of Institute of Tropical Aquaculture hatchery, Universiti Malaysia Terengganu. Crabs were classified mature according to the shape of abdominal flap and carapace size according to the study by Ikhwanuddin et al. (2011). Crab carapace width (CW) and body weight (BW) were recorded at the start of experiments. Widths of crabs (CW) are the distance between the two tips of the 9<sup>th</sup> spine of anterolateral carapace was measured using digital caliper (accuracy 0.01 mm, Mitutoyo, USA).

**Experimental design.** The crabs were kept in 2 ton fiberglass tanks (1.2 × 3.0 × 0.6 m) and wet body weight (BW) was measured using digital balance (accuracy 0.01 g, Shimadzu, Japan). Experiment was divided into two treatments: Treatment A of 60 crabs fed with blood cockle, *Anadara granosa* and Treatment B of 60 crabs fed with fish, *Decapterus* spp. Crabs were fed at 10% biomass feeding rate, twice daily. Water quality was maintained at 15-17 ppt and 29-30°C with 100% water exchanged daily. Experiment last for 60 days and sampling was done at every 10 days. Ten crabs of each treatment were dissected at every sampling to observe the ovarian development. Any crabs left after day 60 were dissected to observe the maturity of the ovary. For initial observation experiment, another 120 crabs with 30 crabs for four ovarian maturation stages were also sampled every week from the study site used to described and recorded the ovarian maturation stages of wild caught *S. olivacea*, which is referred as control. The selection of the crab's size was done randomly for each treatment.

**Histological examination** The control (wild caught crabs) and laboratory sampled (cockle-fed crabs and fish-fed crabs) of crabs at every ovarian maturation stage were sacrificed and the ovaries were taken to perform a histological analysis. The tissue samples were fixed in 10% formaldehyde with replacement of distill water with seawater and processed by standard histological techniques and stained with hematoxyline and eosine (HE).

**Data collection and analysis** All 240 crabs used for control and treatments were measured for their body weight (BW) and Gonadosomatic Index (GSI). Crab ovaries of all crabs were weight to determine the GSI. GSI was expressed as the percentage of the ovarian weight (OW) relative to the BW using formula as follows:

The diameter of at least 30 oocytes per crabs was measured for every crab samples used in the present study. The mean of GSI, oocytes diameter between each ovarian maturation stages of wild caught crab, cockle-fed crab and fish-fed crab were compared using analysis of variance (ANOVA) followed by Tukey Test to find the different between mean.

#### PROXIMATE COMPOSITION ANALYSIS OF DIETS

Diets used in the present study were analyzed for proximate composition according to the AOAC (2002) and analyses of lipid extraction were carried out using same international standard methods. Meanwhile, moisture was determined using the air oven method (100°C for 18 h; AOAC 950.46B), ash using the basic heating technique (550°C for 5 h; AOAC 920.15), and crude protein was determined by the nitrogen combustion procedure (AOAC 992.15).

#### RESULTS

##### HISTOLOGICAL EXAMINATION

**Wild caught crabs.** Immature ovary was filled with oogonia, follicle cells and primary oocytes. Oocytes were surrounded by follicle cells (Figure 1). In early maturing ovary, follicle cells still can be observed surrounding the oocytes and the small yolk globules appeared in the cytoplasm of larger oocytes (Figure 2). Yolk globules obviously appeared in the cytoplasm of the oocytes but follicles cells were hardly recognized in the pre-maturing (Figure 3). In the fully matured, cytoplasm of the oocytes was filled with very large and fused yolk globules which can be used as the indicator of this stage. Follicle cells were hardly observed and nucleus was barely visible (Figure 4). **Captive crabs fed with different diets.** From the histological observation, immature ovary of cockle-fed and fish-fed crabs was filled with oogonia, primary oocytes and follicle cells. Primary oocytes observed to have large nuclei and were surrounded by follicle cells. During early maturing ovarian maturation, histological observation showed that follicle cells still can be observed and there was also appearance of small yolk globules in the cytoplasm of larger oocytes of both treated crabs. Size of nucleus

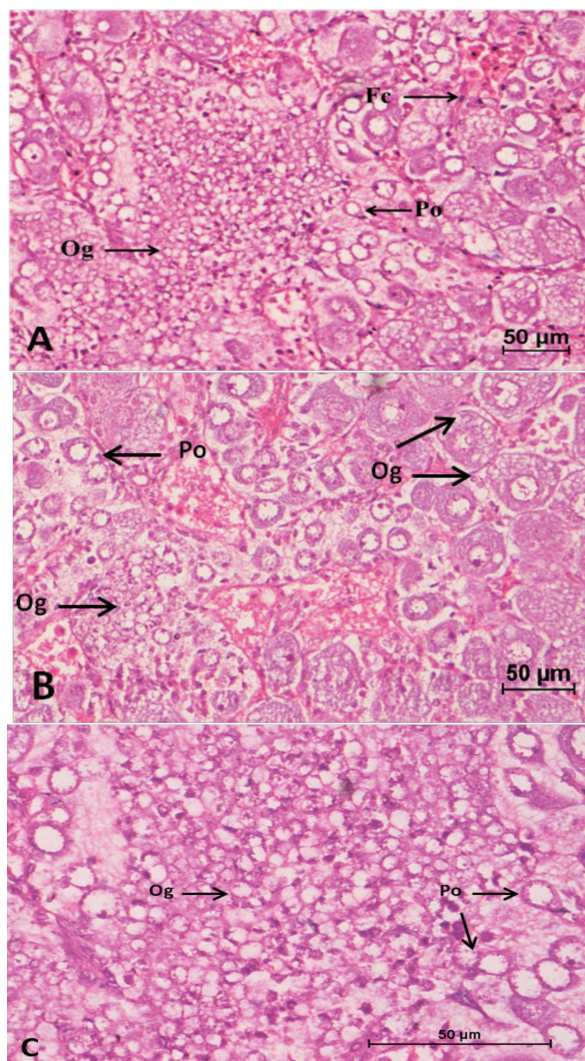


FIGURE 1. Histological section of ovary stage 1 of (A) wild caught, (B) cockle-fed crab and (C) fish-fed crab of *Scylla olivacea* showing oöginia (Og), follicle cells (Fc) and primary oocytes (Po) in each lobe. The follicle cells surround the larger oocytes with oocyte contain a large nucleus (N)

was large at this stage of maturation. Meanwhile, in pre-maturing, the histological examination showed that large and obvious yolk globules occurred in the cytoplasm of the oocytes. During this stage, follicle cells started flattened and size of nucleus was decreased and shrinks. Histological observation during fully matured showed that yolk globules observed fused to each other. Nucleus was flattened, smaller and was barely visible as compared to the previous stage.

#### GONADOSOMATIC INDEX

Wild caught crabs. GSI remained low during the earlier stage with mean GSI of  $2.46 \pm 1.31\%$  and the highest GSI recorded was in class of 1-5% with frequency of 86.67% (Table 1). The volume of ovary increases but not significantly differ ( $p < 0.05$ ) from immature as been shown by GSI (Table 2). The volume of pre-maturing

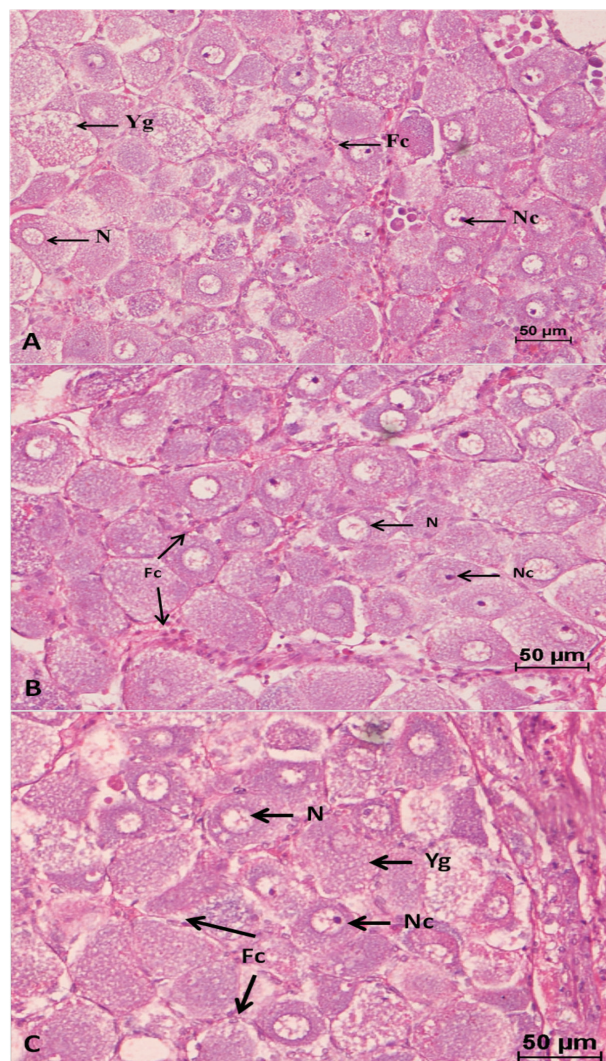


FIGURE 2. Histological section of ovary stage 2 of (A) wild caught, (B) cockle-fed crab and (C) fish-fed crab of *Scylla olivacea* showing yolk globule (Yg) start to appear in the cytoplasm of advance oocyte. Follicle cells (Fc) still observed during this stage. Size of nucleus (N) was still large and nucleolus (Nc) obviously observed

ovary increased significantly ( $p < 0.05$ ) and much heavier than the immature as been shown by mean GSI (Tables 1-2). GSI was also significantly ( $p < 0.05$ ) increased from pre-maturing to fully matured with mean GSI of  $10.71 \pm 4.29\%$  (Tables 1-2). A fully matured ovary was classified to have more than 10% GSI with frequency of 46.67% (Tables 1-2).

Captive crabs fed with different diets. GSI of *S. olivacea* remained low for both cockle-fed and fish-fed crabs during immature ovary (Table 2). Both treated crabs were classified to have GSI below than 1% with frequency of 95.24% and 100.00% (Table 2). Volume of the early maturing ovary of cockle-fed and fish-fed crabs increased obviously. During early maturing ovary, mean GSI of fish-fed crabs was significantly higher ( $p < 0.05$ ) as compared to mean GSI of cockle-fed crabs and wild crabs. Pre-maturing ovary of cockle-fed and fish-fed crab increasing in volume and start to cover the

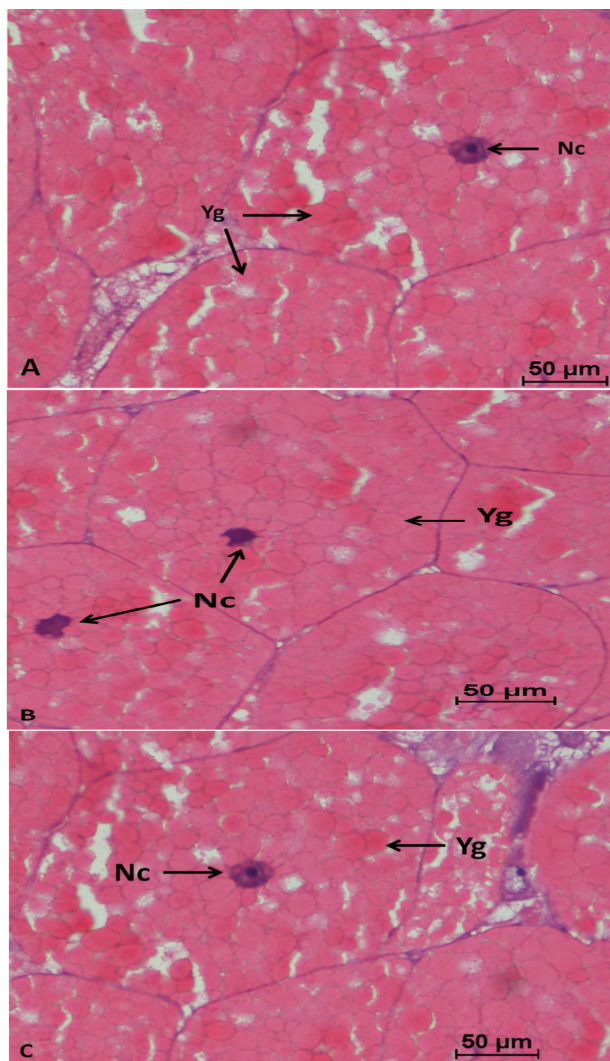


FIGURE 3. Histological section of ovary stage 3 of (A) wild caught, (B) cockle-fed crab and (C) fish-fed crab of *Scylla olivacea* showing the decreasing size of nucleus (N). Yolk globules (Yg) also increased and filled the cytoplasm of oocyte

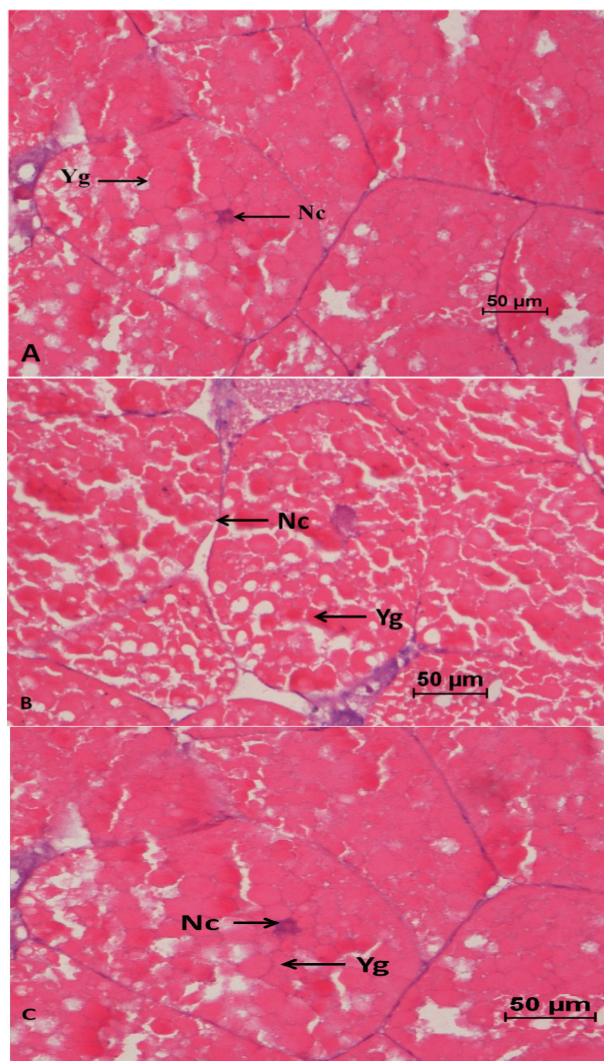


FIGURE 4. Histological section of ovary stage 4 of (A) wild caught, (B) cockle-fed crab and (C) fish-fed crab of *Scylla olivacea* showing larger oocytes with large yolk globules (Yg) fused to each other and occupying the entire cytoplasm. Nucleolus (Nc) is barely visible

TABLE 1. Mean frequency percentage and gonadosomatic index at various ovarian maturation stages of wild caught mud crab, *Scylla olivacea*

Gonadosomatic index classification (%)	Mean frequency percentage (%)			
	Stage 1	Stage 2	Stage 3	Stage 4
<1	13.33	13.33	3.33	0
1-5	86.67	66.67	26.67	16.67
6-10	0	20	53.33	36.67
>10	0	0	16.67	46.67
Number of sample	30	30	30	30

\*Means with the same superscript in the same row are not significantly different ( $p>0.05$ ).

hepatopancreas. Individual eggs of both treated crabs were visible to naked eye. During pre-maturing ovary, mean GSI of fish-fed crab was significantly higher ( $p<0.05$ ) as compared to mean GSI of cockle-fed crab and wild caught crab. During fully matured ovary of cockle-fed crabs, mean GSI recorded was  $10.20\pm 3.74\%$  (Table 2), not

significantly different ( $p<0.05$ ) from wild caught crabs. The highest mean GSI during this stage for cockle-fed crab was classified in class of more than 6-10% with frequency of 75.00% (Table 2). There was no record on the fully matured ovary of fish-fed crab due to no fully matured ovary was produced during 60 days study period.

TABLE 2. Gonadosomatic index classification of stage 1 to stage 4 ovary of *Scylla olivacea* fed with different diets

GSI classification (%)	Stage 1			Stage 2			Stage 3			Stage 4		
	(Frequency percentage - %)			(Frequency percentage - %)			(Frequency percentage - %)			(Frequency percentage - %)		
	Wild crab	Cockle-fed crab	Fish-fed crab	Wild crab	Cockle-fed crab	Fish-fed crab	Wild crab	Cockle-fed crab	Fish-fed crab	Wild crab	Cockle-fed crab	Fish-fed crab
<1	13.33	95.24	100.00	13.33	29.41	0.00	3.33	0.00	0.00	0.00	0.00	0.00
1-5	86.67	4.76	0.00	66.67	58.82	13.04	26.67	21.43	0.00	16.67	0.00	12.50
6-10	0.00	0.00	0.00	20.00	11.76	47.83	53.33	71.43	38.10	36.67	38.10	75.00
>10	0.00	0.00	0.00	0.00	0.00	39.13	16.67	7.14	61.90	46.67	61.90	12.50
Number of samples	30	21	16	30	17	10	30	14	23	30	8	8
Mean GSI index (%)	2.46±1.31 <sup>a</sup>	0.66±0.98 <sup>b</sup>	0.50±0.11 <sup>b</sup>	3.46±2.29 <sup>a</sup>	2.22±1.74 <sup>a</sup>	10.06±3.53 <sup>b</sup>	7.65±3.50 <sup>a</sup>	7.96±3.48 <sup>a</sup>	11.53±3.08 <sup>b</sup>	10.71±4.29 <sup>a</sup>	10.20±3.74 <sup>a</sup>	10.20±3.74 <sup>a</sup>

<sup>a</sup>Means with the same superscript in the same row at different ovarian maturation stages (stage 1-4) are not significantly different ( $p>0.05$ ), GSI: gonadosomatic index, no stage 4 ovary for fish-fed crab was produced during 60 days study period

## OOCYTE SIZE

Wild caught crabs. From the histological observation, the oocytes size during all stages of ovarian maturation of wild caught crabs was significantly different ( $p < 0.05$ ) (Table 3). In Early maturing ovary, oocytes diameter significantly increased ( $p < 0.05$ ) with mean diameter of  $81.87 \pm 6.01 \mu\text{m}$  (Table 3). The size of oocytes during pre-maturing ovary increased rapidly and significantly different from the previous ovary early maturing (Table 3). Fully matured ovary of wild caught *S. olivacea* was recorded to have very large oocytes with mean oocyte diameter of  $177.63 \pm 11.35 \mu\text{m}$  (Table 3).

Captive crabs fed with different diets. The oocytes size during immature ovary of cockle-fed and fish-fed crabs was significantly different ( $p < 0.05$ ) (Table 3). The mean oocyte diameter of fish-fed crabs recorded was  $68.74 \pm 8.81 \mu\text{m}$ , significantly larger ( $p < 0.05$ ) as compared to the mean oocyte diameter of cockle-fed ovary. During early maturing ovarian maturation, the mean oocyte diameter of cockle-fed crabs was significantly larger ( $p < 0.05$ ) as compared to the fish-fed crabs (Table 3). The size of oocytes during pre-maturing ovary of both cockle-fed and fish-fed crabs increased rapidly and significantly different ( $p < 0.05$ ) from the previous stage. Mean oocyte diameter of fish-fed crab ovary was significantly larger ( $p < 0.05$ ) as compared to cockle-fed crab ovary (Table 3). Fully matured ovary of cockle-fed crabs had mean oocyte diameter of  $282.38 \pm 55.52 \mu\text{m}$ , significantly larger ( $p < 0.05$ ) from the previous stage. Mean oocyte diameter of cockle-fed crabs was significantly larger ( $p < 0.05$ ) as compared to wild crab (Table 3). There was no record for the oocyte diameter of fully matured ovary of fish-fed crabs from this study. This was due to no fish-fed crab produced fully matured ovary during 60 days of study period.

## PROXIMATE COMPOSITION

Proximate analysis of both diets showed that, percentage of protein is quite similar ( $17.35 \pm 0.13\%$  for fish and  $15.99 \pm 0.00\%$  for cockle), except the content of lipid,

which was higher in fish and lower in cockle ( $4.4 \pm 2.56\%$  for fish and  $1.93 \pm 1.28\%$  for cockle) or fat ( $1.35 \pm 0.81\%$  for fish and  $1.51 \pm 0.51\%$  for cockle). Table 4 showed the result of full proximate compositions of the diets used in the present study.

## DISCUSSION

Ovarian maturation stages of *S. olivacea* were classified into four stages of immature, 2, 3 and 4. This classification was correlated closely with the mean GSI and mean oocyte size. As the ovary mature from immature to fully matured, the volume of ovary was also increased as been shown by the increment of mean GSI and mean oocyte size in the present study. A common indicator that can be observed morphologically during ovarian maturation stages of crustacean is the increment of ovary volumes. During ovarian development, yolks accumulate in the hepatopancreas and transported into oocytes (Ravid et al. 1999) as a process of nutrients enrichment into the ovary which required during embryonic and larval development (Quinitio et al. 2007). These accumulations affected morphology of the ovary as been observed on the increment of mean GSI value. Histological observation of present study showed that, during early ovarian maturation stages (immature and early maturing), there were many primary oocytes and the follicle cells observed surrounds the oocytes. The follicle cells and primary oocytes observed flattened during further maturation stages of *S. olivacea* (pre-maturing and fully matured). At the same time, the size of nucleus also large during the early stages and decreased during further maturation stage. The oocyte size of *S. olivacea* also significantly increased as the maturation of ovary progressed. These results are similar to those observed in gonad of mud crab, *S. serrata* (Forskål 1775) (Quinitio et al. 2007), mud crab, *S. olivacea* (Islam et al. 2010a) and mud crab, *S. paramamosain* (Estampador 1949) (Islam et al. 2010b). The present of many follicle cells and large nucleus of the oocytes in the immature and early

TABLE 3. Oocyte diameter at various ovarian maturation stages of *Scylla olivacea* fed with different natural diets

Treatment	Mean oocyte diameter ( $\mu\text{m}$ )			
	Stage 1	Stage 2	Stage 3	Stage 4
Cockle-fed crab	$64.60 \pm 8.99^a$	$70.99 \pm 9.52^b$	$206.16 \pm 29.77^c$	$282.38 \pm 55.22^d$
Fish-fed crab	$68.74 \pm 8.81^a$	$68.88 \pm 9.18^b$	$230.23 \pm 19.88^c$	n/a
Wild caught crab	$70.89 \pm 7.75^a$	$81.87 \pm 6.01^b$	$139.14 \pm 14.06^c$	$177.63 \pm 11.35^d$

\*Means with the same superscript in the same rows are not significantly different ( $p > 0.05$ ), n/a: not available

TABLE 4. Proximate analysis of different natural diets used in the present study

Sample	Moisture	Ash	Crude protein	Crude lipid	Fat
	Mean percentage (%)				
Fish, <i>Decapтерus</i> spp.	$75.70 \pm 1.96$	$1.65 \pm 0.57$	$17.35 \pm 0.13$	$4.40 \pm 2.56$	$1.35 \pm 0.81$
Blood cockle, <i>Anadara granosa</i>	$78.94 \pm 2.18$	$1.63 \pm 0.34$	$15.99 \pm 0.00$	$1.93 \pm 1.28$	$1.51 \pm 0.51$

maturing of *S. olivacea* indicate that the early stages of ovarian maturation are involved the yolk synthesis has been reported in other crustacean such *S. olivacea* (Islam et al. 2010a), *S. paramamosain* (Islam et al. 2010b) and mantis shrimp, *Oratosquilla ratoria* (De Haan, 1844) (Kodama et al. 2004). Yano and Chinzei (1987) suggested that, follicle cells are responsible in the synthesis of vitellogenin, the precursor of the major egg yolk protein in kuruma prawn, *Penaeus japonicus* (Spence Bate 1888).

As the ovary matured from immature to fully matured, the follicle cells and size of nucleus flattened. The size of nucleus obviously decreased and shrinking after reaches pre-maturing and barely visible at fully matured maturation. According to Yamada et al. (2007), this pattern indicates the onset of germinal vesicle breakdown. On the other hand, *in vitro* study by Semenkov et al. (2006) showed that, the final oocyte maturation in fish involved the breakdown of the germinal vesicle and followed by the ovulation. The vitellogenesis during ovarian maturation of mud crab, *S. serrata* is divided into two phase, primary vitellogenesis and secondary vitellogenesis. The beginning of the secondary vitellogenesis is characterized by the rapid increment oocytes and the mass accumulation of yolk body within the oocyte. Immature and early maturing ovarian maturation of *S. olivacea* observed contained many follicle cells surrounding the oocytes membrane of primary oocytes and advanced oocyte with yolky substances. In the present study, it is suggested that, immature and early maturing ovaries can be classified as primary vitellogenesis. Pre-maturing and fully matured ovarian maturation of *S. olivacea* can be best classified as vitellogenic phase or secondary vitellogenesis according to the rapid increment of oocyte size. At the same time, the cytoplasm of pre-maturing and fully matured oocytes was filled with yolk globules and germinal vesicle breakdown occurred indicates the maturity of the oocytes. Deposition, transportation and accumulation of nutrients into the ovaries during vitellogenesis not only affected the fatty acids composition, but also the ovary cells size. This can be observed from the present study where the oocytes size increased significantly during ovarian maturation of *S. olivacea*. Significant increment in oocyte size and accumulation of yolk proteins were the characters during the ovarian maturation of crustaceans.

It is suggest that, a rapid increase in the oocyte size during ovarian maturation stages of *S. olivacea* is appeared to be closely linked with the vitellogenesis. Vitellogenesis involved the synthesis and uptake of vitellogenin from the hemolymph into oocytes accompanied by the substantial quantities of yolk and other nutrients accumulation within the oocytes. Vitellogenin is the protein synthesized outside the ovary and transported to the ovaries, which subsequently involved in the synthesis of egg yolk protein and vitellin (Hagedorn & Kunkel 1979). The main constituents of yolk are proteins and lipids. Lipids are insoluble in water and form a complex with protein known as lipoprotein. Vitellin is the major lipoprotein that accumulates within the ovaries during vitellogenesis

(Chen et al. 1999; Ravid et al. 1999). As been mentioned earlier, yolk protein accumulation is important process, which subsequently serves as the sources of nutrition required by the developing embryo (Quinitio et al. 2007). Histologically, an active yolk deposition can be observed within the matured and advanced oocyte of most crustaceans, including *S. olivacea* of present study. It is suggested that, diet contain high lipid composition influenced the production of ovary. Dietary lipid plays an important role as potential supplier of energy, essential fatty acids and fat soluble vitamins. At the same time, dietary lipids also affect the quality of cultured fish and crustacean broodstock. The ovarian maturation in portunid crabs are largely depend on the variation of fed given during their gonad development. The use of natural food usually lack of essential dietary fatty acids and directly reduces the reproductive maturation in portunid crabs (Azra & Ikhwanuddin 2016). On the other hand, fresh natural food such as squid and shrimp showed fast maturation when it fed to the *S. serrata* broodstock (Pattiasina et al. 2012).

In conclusion, the ovarian maturation stages of *S. olivacea* had been recorded and described based on the histological characteristics. The present study has classified four different stages during the ovarian maturation stages of *S. olivacea* from Malaysian waters (immature, early maturing, pre-maturing and fully matured). The color and status of the ovary of mud crab are closely related to its cellular development (e.g. oocyte size and gonadosomatic index). Ovarian biopsy technique can be used together with information from present study to determine the ovary status of mud crab without killing the crab. Present study showed that fish-fed crabs produced high volume of ovary as compared to the cockle-fed crab and wild caught crabs. At the same time, cockle-fed crab matured faster as compared to the fish-fed crab. For a better result, it is suggest that, a combination diets between blood cockle, *Anadara granosa* and fish, *Decapтерus* spp. as well as the other natural diets contain high fatty acid, should be given to the *S. olivacea* during maturation program. The classification of ovarian maturation stages provides the baseline information for further studies on the reproductive biology of *S. olivacea*. Likewise, the information provides a guide for broodstock management in the hatchery such as determination of the optimum diet for the production of mature crabs, which sometimes very hard to produce in captivity. At the same time, this information can help in reduce time and cost of maintenance in maturation tanks. When this information available, it would enables crabs growers, aquaculturists including fishermen to choose the best diet for mud crab maturation as well as in choosing the right broodstock for maturation program.

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Azmie Ghazali, Ambok Bolong Abol-Munafi, Mohamad N. Azra & Mhd Ikhwanuddin\*  
Institute of Tropical Aquaculture  
Universiti Malaysia Terengganu  
21030 Kuala Terengganu, Terengganu Darul Iman  
Malaysia

Noordiyana Mat Noordin  
School of Fisheries and Aquaculture Sciences  
Universiti Malaysia Terengganu  
21030 Kuala Terengganu, Terengganu Darul Iman  
Malaysia

\*Corresponding author; email: ikhwanuddin@umt.edu.my

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