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Original Article

Association of oocyte development with ovarian morphology and gonadosomatic index in the sesarmid crab *Episesarma singaporense* (Tweedie, 1936)

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Abstract

The sesarmid crab *Episesarma singaporense* is an economically important crab in Thailand. In this study, we associated the integrated classification of oocyte development with ovarian morphology and the gonadosomatic index (GSI). All specimens were collected in October 2018 and January – February 2019. The germinative centers of the ovary contained oocytes in oogonia proliferation and primary growth phases (previtellogenic stage; Oc1), whereas peripheral germinal zones contained those in the secondary growth phase: early vitellogenic stage (Oc2), late vitellogenic stage (Oc3), and mature stage (Oc4). The developing ovary was classified into four stages. Stage I ovary (immature stage, GSI = 0.17±0.09, n=58) was characterized by translucent color and oocytes at the oogonia proliferation and primary growth phases. Stage II ovary (developing stage; GSI = 0.59±0.09, n=49) had creamy white color and mainly contained Oc2 oocytes. In Stage III (developed stage; GSI = 1.38±0.09, n=62), ovary color turned into bright orange and Oc3/Oc4 oocytes were prominently observed. Stage IV ovary (mature stage; GSI = 2.54±0.46, n=84) showed deep yellow to red/orange color and was dominantly composed of mature oocytes. These data provide useful information to estimate the oocyte development from ovarian morphology.

Keywords: estuarine area, female reproductive system, histology, oocyte, Sesarmid crabs, Thailand

1. Introduction

Morphological features are important for understanding the decapod reproductive system, which directly contributes to the sustainability of natural stocks (Balasubramanian & Suseelan, 1998; Castiglioni, Negreiros-Fransozo, Greco, Silveira, & Silveira, 2007; Komm & Hinsch, 1987, Stewart *et al.* 2007). In particular, the oocyte differentiation process has been actively investigated in economically important species (Adiyodi, 1985; Balasubramanian & Suseelan, 1998; Komm & Hinsch, 1987; Kulkarni, Glade, & Finguerman, 1991; Mota & Tomé, 1965). In decapods, there have been at least two different criteria for classification of oocyte differentiation processes: cellular features such as cell diameter and nuclear appearance (Mota & Tomé, 1965) and the degree of vitellogenesis (Kulkarni *et al.*, 1991). Based on cellular features, the oocyte differentiation process is typically classified into five stages. For example, oocyte differentiation process of the blue swimmer crab *Portunus pelagicus* and the serrated mud crab *Scylla serrata* has been classified into

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oogonia (Og), Oc1, Oc2, Oc3 and Oc4 (Stewart *et al.* 2007). On the other hand, based on the vitellogenin uptake, oocyte differentiation process has been classified into seven stages: Og, primary oocytes, early previtellogenic oocyte, late previtellogenic oocyte, early vitellogenic oocyte, late vitellogenic oocyte and mature oocyte (Sharifian, Kamrani, Safaie, & Sharifan, 2015). These classifications, however, have not been very consistent. Integrating these classifications with clear criteria and ovarian external morphology enables easy monitoring of decapod reproductive cycle, which contributes to the establishment of successful aquaculture.

Episesarma singaporense (Tweedie, 1936), locally known as the "sesarmid crab" in Thailand, is a key species in the mangrove ecosystem and is one of the most economically important crabs for human consumption (Teinsongrusmee, 2009). In Thailand, the market demand of *E. singaporense* reaches 18,000 million tons per year; however, the annual production of this crab is only about 12,000 million tons per year (Teinsongrusmee, 2009). Unfortunately, aquaculture of this crab has not been successful at the industry level, and overfishing associated with reducing recruitment rate is a hot issue. The sesarmid crab is therefore a target species of the stock enhancement program in Thailand. Under this circumstance, the pilot *E. singaporense* culture at the Marine Crab Research Laboratory, Faculty of Science and Fisheries Technology, Rajamangala University of Technology Srivijaya, Thailand (Sudtongkong, Decharun, & Watttanagul, 2014), achieved certain success, but their growth and survival rates remained low. This situation might be due to poor maternal nutrition (e.g., imbalanced essential fatty acids) during the gonadal devolvement (Wu *et al.* 2010). Subsequent success of the sesarmid crab aquaculture depends at least partly on accurate estimation of its gonadal development.

In this research, we report detailed ovarian histology of the sesarmid crab *E. singaporense*, proposing an integrative classification with clear histological criteria. We also attempted to develop a method to assess the reproductive state of *E. singaporense* from external morphology by associating the histological observation with ovarian morphology and gonadosomatic index (GSI).

2. Materials and Methods

2.1 Sesarmid crab collections and study area

We analyzed a total of 303 female *E. singaporense* caught from the estuarine area in Palian District, Thailand (7º 08' 58.12" N, 99º 40' 10.11" E), during October 2018 and January – February 2019. This area is surrounded by big mangrove forests and is considered to be the habitat of the sesarmid crab in Thailand. The sesarmid crab was identified according to the taxonomic guideline of Lee, Ng & Ng. (2015). The temperature and the salinity of the area were 23–28 °C and 20–45 ppt, respectively. Following the capture, all live crabs were air-lifted and transported to the Marine Science Laboratory at Department of Marine Science, Faculty of Science and Fisheries Technology, Rajamangala University of Technology Srivijaya, Trang Campus, Thailand. The experiment was approved by the Animal Care and Use Committee of Rajamagala University of Technology Srivijaya (ID#IAC 02-01-62).

2.2 Ovarian morphology and gravimetric analyses

All sesarmid crabs were euthanized by a rapid cooling method for 30 minutes (Wilson *et al.* 2009). Body weight (BW) and carapace width (CW) were determined with scales and calipers, respectively. For the determination of the ovarian morphology, the dorsal part of the carapace was opened and observed using a digital camera (Canon EOS 550D). Then the ovaries were dissected and weighted for the calculation of the gonadosomatic index (GSI = gonadal weight/total weight x 100).

2.3 Histological observations

A small portion of ovarian tissues was fixed in Davidson's fixative (330 ml 95% ethyl alcohol, 220 ml 100% formalin, 115 ml glacial acetic acid and 335 ml distilled H_2O; Dietrich and Krieger, 2009) for 48 h at room temperature. The fixed tissues were then processed using a standard histological technique (Presnell & Schreibman, 1997; Suvarna, Layton, & Bancroft, 2013). The paraffin blocks were cut at 4 µm of thickness and then stained with Harris's hematoxylin and eosin (H&E). Some sections were histochemically stained with Masson's trichrome (MT), cresyl violet (CV) and periodic acid-Schiff (PAS). The ovarian structure, oogenesis and its development of all sesarmid crabs were determined with a Leica DM750 light microscope (Boston Industries, Inc; U.S.A.). The size of the oocytes was measured from three sections per samples (about 50 cells per section at magnifications of 40x for oognium and 10x for other oocyte stages) and represented as mean ± SD. To avoid the redundant measurement, the interval of each section was set to more than 400 µm, which was larger than the average size of oocytes in any stages (see Results; the largest average oocyte size was 168.09±1.04 µm in this study).

3. Results and Discussion

3.1 Ovary structure and oogenesis

In this study, we determined the ovarian morphology of the sesarmid crab, proposing a novel integrated classification of the oocyte differentiation process that includes three distinct stages and their subdivisions: oogonial proliferation, primary growth phase [previtellogenic oocyte (Oc1)], and secondary growth phase [early vitellogenic oocyte (Oc2), late vitellogenic oocyte (Oc3) and mature oocyte (Oc4)].

Cellular features such as cell size, nuclear characteristics, cytoplasmic properties and follicular cell characteristics were used for the classification.

The ovary of *E. singaporense* was surrounded by a thin single layer of ovarian wall (Figures 1A-B). Each lobe consisted of two zones, the germinal center (sometimes called germinative center) and germinal zone (Figure 1A). The germinal center was defined as the center area of the ovarian lobe with an irregular shape containing oocytes in oogonia proliferation and primary growth stages (Figure 1C), as previously reported for the testicular germinal center containing proliferating spermatogonia (Moyano, Gavio, & Cuartas, 2010). Oocytes at the secondary growth stages were located in

germinal zone, the peripheral region of the ovarian lobe (Figures 1D-H). Therefore, the size of germinal center decreased during the ovarian maturation process. The ovary contained oocytes at various differentiation stages in the ovarian lobes, and therefore was considered to be the asynchronous developmental ovary. Below we describe characteristics of oocytes in each differentiation stage.

Oogonia: Oogonia had a spherical or slightly ovoid shape and a mean diameter of 10.53±0.91 µm (Figure 1C). Numerous euchromatin and small blocks of heterochromatin were observed in a scattered pattern in the nucleoplasm (Figure 1C). A thin layer of basophilic cytoplasm was observed (Figure 1C).

Pre-vitellogenic oocyte (Oc1): Oocytes in the pre-vitellogenic stage showed evidence of meiosis, and their mean diameter increased to 21.23±1.32 µm. The ooplasmic basophilia during early pre-vitellogenic stage was observed in this stage (Figure 1C) as reported in other decapods including the green tiger prawn *Penaeus semisulcatus*, the black tiger shrimp *P. monodon* (Ongvarrasopone *et al.*, 2006) and the kuruma shrimp *Marsupenaeus japonicus* (Okumura *et al.*, 2006). The basophilic homogeneous cytoplasm is related to the formation of organelles such as Golgi complexes, endoplasmic reticulum and ribosomes (Komm & Hinsch, 1987; Wilder, Okumura, & Tsutsui, 2010). Krol *et al.* (1992) reported that the process of pre-vitellogenesis is associated with increased activity of various cytoplasmic organelles, which results in basophilic cytoplasm. Subsequently, lipid droplets became apparent in the late pre-vitellogenic stage (Figure 1D) as reported in many other decapods (Chen *et al.*, 2004; Walker *et al.*, 2006). In parallel, increased cell size of about 65.09 ± 1.22 µm in diameter was observed. These lipid droplets function as a nutrient reservoir for the embryonic development (Chen *et al.*, 2004; Walker *et al.*, 2006). The development of follicle cell layers was also observed (Figure 1D-1E).

Early vitellogenic oocyte (Oc2): This stage was characterized by the formation of yolk granules (H&E method, Figure 1D), which was also detected by the PAS staining (data not shown). The yolk granules are considered to contain glycoproteins as a major source of energy for embryos (Kulkarni *et al.*, 1991; Lee & Watson, 1995). The cell size increased compared to the pre-vitellogenic stage with a mean diameter of 89.07±0.98 µm. At the same time, the cytoplasm lost the basophilic characteristics and became slightly acidophilic (Figure 1E). This observation is consistent with previous reports (Fainzilber, Tom, Shafir, Applebaum, & Lubzens, 1992; Riley & Tsukimura, 1998; Zara, Gaeta, Costa, Toyama, & Caetano, 2013). Many lipid droplets were observed at the peripheral part of the cell and negatively stained by the H&E staining (Figure 1D). The nucleus was still visible, but the shape became irregular (Figure 1E). The well-defined follicular cells surrounded the oocyte (Figure 1D). We further confirmed using cresyl violet staining that the follicular cell layer had the oval nuclei stained in blue-violet color (data not shown). The considerable increase in the number of follicular cells might be related to the uptake of yolk granules, which was supported by several investigators (Adiyodi & Subramonian, 1983; Ravi, Manisseri, & Sanil, 2013).

Late vitellogenic oocyte (Oc3): The mean diameter of the cell increased to 150.44±0.96 µm in this stage. The yolk granules were more evident, being approximately 10-15 µm in diameter (Figures 1E-1H). The cytoplasm became strongly acidic in H&E staining (Figure 1G) and showed a pinkish color in the PAS staining (Figure 1G), as reported in other decapods including the giant river praw *M. rosenbergii* (Mee ratana & Sobhon, 2007), the blue swimmer crab *P. pelagicus* and the serrated mud crab *S. serrata* (Stewart *et al.*, 2007). The nucleus decreased the size and showed an irregular shape during the late vitellogenic stage (Figure 1F). The follicular cell layers were still observed with a well-differentiated layer (Figure 1F); however, the nuclei of this cell were changed into a flat shapes (Figure 1H). This might be associated feature with the end of vitellogenesis, in which the function of nucleus declines. While this phase has been sometimes classified as Oc4 (Stewart *et al.* (2007), we described

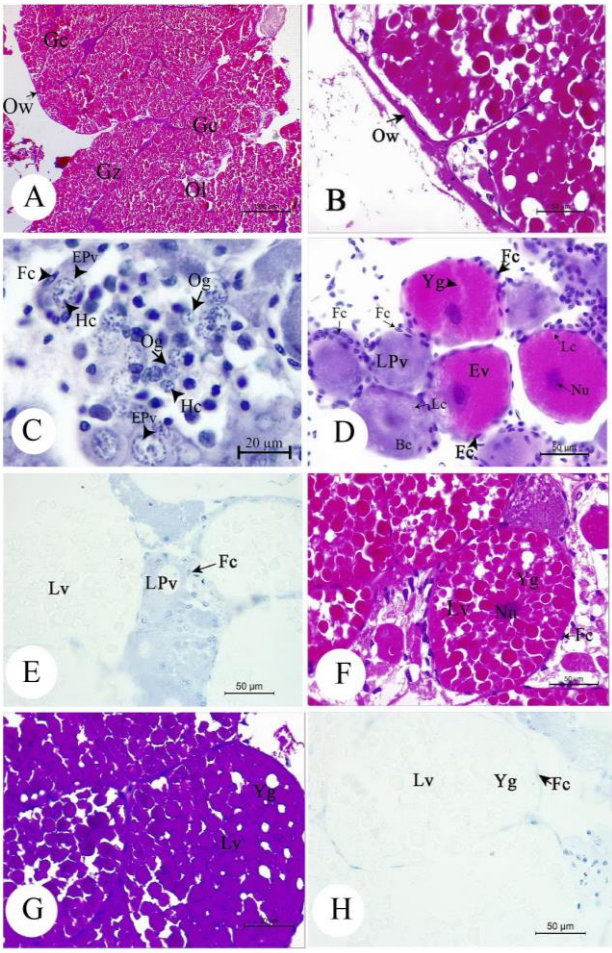
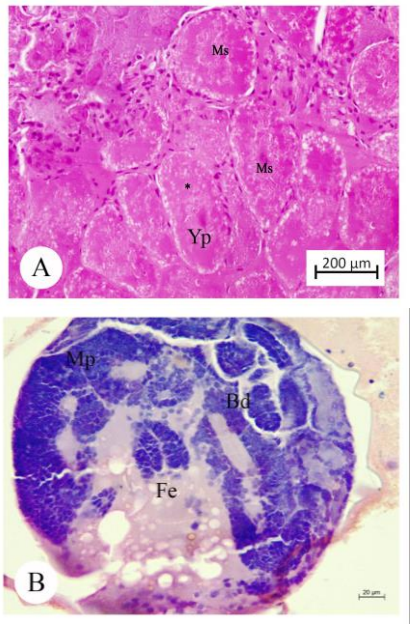


Figure 1. Light photomicrographs of developed ovary and oogenesis in the sesarmid crab, *Episesarma singaporense*. A,B) Ovarian lobe (Ol) was classified into germinal center (Gc) and germinal zone (Gz), which was surrounded by a thin layer of ovarian wall (Ow). Harris hematoxylin & eosin staining method. Scale bars: A, 500 µm; B, 50 µm. C-H) Enlarged views of differentiating oocytes. C, D, F = Harris hematoxylin & eosin staining; E, H = cresyl violet staining, G = Periodic's Schiff staining. Abbreviations: (Og), pre-vitellogenic stage (Pv), Early vitellogenic stage (Ev), late vitellogenic stage (Lv). Bc = basophilic cytoplasm, Fc = follicular cells, Hc = heterochromatin, Ld = lipid droplets, Nu = nucleus, Yg = yolk granules.

these oocytes as Oc3 following Castiglioni *et al.* (2007) and Stewart *et al.* (2007) (see Oc4 for details).

Mature oocyte (Oc4): The cell reached the maximum size with a mean diameter of 168.09±1.04 µm (Figure 2A). The cytoplasm was completely fused, which is also called "yolk platelet". Nuclei were almost invisible or very small in the section because of the large cell size (Figure 2A). This is a common feature of a fully-grown oocyte in decapods (Castiglioni *et al.*, 2007; Stewart *et al.*, 2007). Fertilized eggs were also observed in the germinal zone (Figure 2B). The fertilized egg was filled with the yolk plate (Figure 2B), whereas the surface of this egg had a typical appearance of well-developed blastodisc eggs (Figure 2B). It was supported by multilayers of blastomeres showing the mesodermal plug (Figure 2B).

Interestingly, fertilized eggs have not been identified in the ovary of crabs in previous literature. The ovary of other spawned crabs is known to return to the spent phase containing prominent Og, Oc1 and follicular cell with some Oc4 remained (Subramoniam, 2017). Once the mature oocytes are fertilized with sperms kept in seminal receptacles, the fertilized eggs are spawned and stuck on the female abdomen (Subramoniam, 2017). This may be a unique reproductive feature of *E. singaporense*, but it is also possible that we observed fertilized eggs accidentally remaining in the ovary since very few fertilized eggs were found in this study. Meanwhile, it is possible that the seminal receptacle of *E. singaporense* may have normal sperm releasing to the ovary for fertilization since the ovary of crabs is closely linked with seminal receptacle via a short oviduct according to a previous report (Mclay & Becker, 2015) about the detailed anatomy of the Infraorder Brachyura.

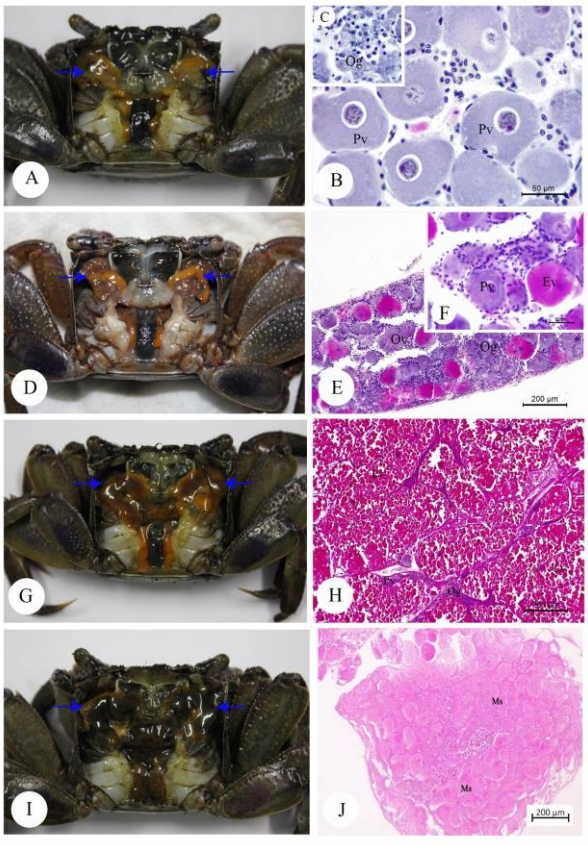


Figures 2. Light photomicrographs of mature ovary containing mature oocyte (A) and fertilized egg (B) of the sesarmid crab, *Episesarma singaporense*. Scale bars: A, 200 µm; B, 20 µm. Abbreviations: asterisk = nucleus, Bd = blastodisc, Fe = fertilized egg, Mp = mesodermal plug, Ms = mature oocyte, Yp = yolk plate. Harris's haematoxylin and eosin (H&E).

3.2 Ovarian morphology

According to the gonadosomatic index (GSI), the ovarian morphology and histological changes in the ovary, the ovarian development of *E. singaporense* was divided into four stages in this study: stage I, II, III, and IV (Figures 3 and Table 1). Percentages of Og and Oc1–Oc4 oocytes in each stages are shown in Figure 4. Similar classification of ovarian morphology was used for the snow crab *Chionoecetes opilio* (Beninger, Lanteigne, & Elner, 1993), the spider crab *Libinia emarginata* (Hinsch & Cone, 1969), the giant river praw *M. rosenbergii* (Meeratana & Sobhon, 2007), the blue swimmer crab *P. pelagicus* (Sumpton, Potter, & Smith, 1994) and the mud crab *S. paramamosain* (Tantikitti, Kaonoona & Pong maneerat, 2015).

Stage I (Immature stage): The ovary was translucent with whitish color, but it was not easy to distinguish the ovary from the digestive structure (Figure 3A). The ovary at this stage contained oogonia (26%) and previtellogenic (Oc1,



Figures 3. Light photomicrographs of the ovarian development in the sesarmid crab *Episesarma singaporense*. A-C: Immature stage, D-F: Developing stage, G-H: Developed stage, I-J: Mature stage. Og = oogonia, Pv = pre-vitellogenic stage, Ev = early vitellogenic stage, Lv = late vitellogenic stage, Ms = mature oocyte stage. Scale bars: B, 50 µm; E, 200 µm. F. 50 µm. H. 200 µm. J. 200 µm. B, E, F, H, J = Harris's haematoxylin and eosin (H&E).

Table 1. Summary of morphology and histology of ovarian development with the average of gonadosomatic index values of *Episesarma singaporense* (Tweedie, 1936).

Stages	Body weight	Carapace width	Ovarian morphology	Histology	GSI
Stage I (Immature)	27.25 ± 3.52	16.78 ± 6.61	Difficult to distinguish from digestive organs Translucent to whitish color	Oogonia and pre-vitellogenic oocytes are dominant in the central zone of the ovarian lobe	0.17 ± 0.09, n = 58
Stage II (Developing)	26.72 ± 2.72	16.74 ± 8.44	Easily distinguished by naked eye Creamy white color	A few oogonia and pre-vitellogenic oocytes are still present, but early vitellogenic oocytes are dominant	0.59 ± 0.15 n = 49
Stage III (Developed)	27.86 ± 2.73	14.75 ± 5.13	Distinct and bright orange	Early and late vitellogenic oocytes	1.38 ± 0.22 n = 62
Stage IV (Mature)	28.78 ± 2.82	17.07 ± 5.74	Deep yellow to red/orange	Ovary is filled with mature oocytes	2.54 ± 0.46, n = 84

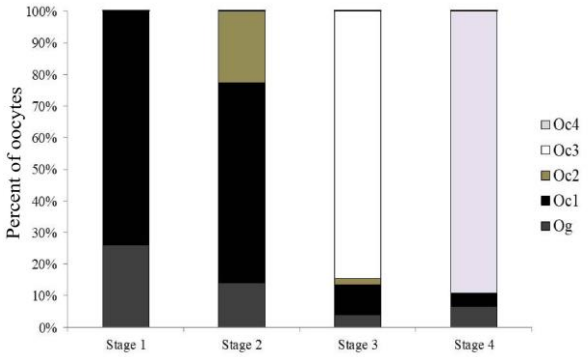


Figure 4. Percentage of oocytes during ovarian development of *Episesarma singaporense* (Tweedie, 1936). Notes: Stage 1 = immature stage, 2 = developing stage, 3 = developed stage, 4 = mature stage, Og = oogonia, Oc1 = pre-vitellogenic stage, Oc2 = early vitellogenic stage, Oc3 = late vitellogenic stage, Oc4 = mature stage.

74%) oocytes in the central zone of the ovarian lobe (Figures 3B-3C).

Stage II (Developing stage): The ovary became creamy white color (Figure 3D). This stage was easily differentiated from the Stage I because of the drastic increase in ovarian size. Few oogonia (14%) and previtellogenic (Oc1, 63.3%) oocytes were present (Figures 3E-3F), while the number of the early vitellogenic (Oc2, 22.66%) oocytes was greatly increased in the germinal zone.

Stage III (Developed stage): The ovary had bright orange color, which covered more than a half of the dorsal hepatopancreas (Figure 3G). The ovaries still contained few oogonia (4%) and previtellogenic (Oc1, 9.33%) oocytes in the germinative center the ovarian lobe (Figure 3H). Few early vitellogenic (Oc2, 2%) oocyte were found, but late vitellogenic (Oc3, 84.66%) oocytes were dominent (Figure 3H).

Stage IV (Mature stage): The ovary at this stage had deep yellow to red/orange color (Figure 3I). The ovary was almost fully filled with mature (93.33%, Oc4) oocytes (Figure 3J). Oogonia (6.66%) and previtellogenic (Oc1, 4%) oocytes were very rarely observed. These characteristics are similar to mature ovaries of some decapods such as the spider crab, *L. emarginata* (Hinsch & Cone, 1969), the mole crab, *Emerita asiatica* (Gunamalai & Subramoniam, 2002), and the mud crab, *Scylla paramamosain* (Cui, Liu, & Chu, 2005).

3.3 Carapace weight (CW) and gonadosomatic index (GSI)

Female specimens of *E. singaporense* showed the highest BW (28.78±2.82 g) and CW (17.07±5.74 mm) in the Stage 4 (Table 1). The GSI were gradually increased from Stage 1 (0.17±0.09%) to Stage 4 (2.54±0.46%) during reproductive cycle (Table 1). GSI and was thus closely associated with the oocyte differentiation stages as reported in the crucifix crab, *Charybdis feriatus* (Nieves, Olfindo, & Macale, 2015).

4. Conclusions

In the present work, we associated oocyte differentiation process, ovarian morphology and GSI in an economically important crab *E. singaporense*. These results provide useful information to determine the reproductive potential in natural and cultured populations. Moreover, fertilized egg were observed in this study, which will be beneficial to understand reproductive biology of the sesar-mid crab *E. singaporense*. Further studies could examine the accurate reproductive cycle from estuarine regions to validate the relationship between gonadal maturation and spawning season of *E. singaporense*.

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References

Adiyodi, R. G. (1985). *Reproduction and its control. The Biology of Crustacea V8* (pp. 147-215). New York, NY: Academic Press.

Balasubramanian, C. P., & Suseelan, C. (1998). Reproductive biology of female deep-water crab *Charybdis smithii* (Brachyura, Portunidae) from the India seas. *Asian Fisheries Science, 10*, 211-222.

Beninger, P. G., Lanteigne, C., & Elner, R. W. (1993). Reproductive processes revealed by spermatophore dehiscence experiments and by histology, ultrastructure, and histochemistry of the female reproductive system in the snow crab *Chionoecetes opilio* (O. Fabricius). *Journal of Crustacean Biology, 13*, 1-16.

Castiglioni, D. D. S., Negreiros-Fransozo, M. L., Greco, L. S. L., Silveira, A. F., & Silveira, S. O. (2007). Gonad development in females of fiddler crab *Uca rapax* (Crustacea, Brachyura, Ocypodidae) using macro and microscopic techniques. *Iheringia, Se´rie Zoologia*, *4*, 505-510.

Cui, Z., Liu, H. & Chu, K.L. (2005). Inhibitory effects of the androgenic gland on ovarian development in the mud crab *Scylla paramamosain*. *Comparative Biochemistry and Physiology - Part A Molecular and Integrated Physiology, 140*, 343-348.

Dietrich, D. R. & Krieger, H. O. (2009). *Histological analysis of endocrine disruptive effects in small laboratory fish*. New Jersey, NJ: John Wiley and Sons.

Fainzilber, M., Tom, M., Shafir, S., Applebaum, S. W., & Lubzens, E. (1992). Is there extraovarian synthesis of vitellogenesis in penaeid shrimp. *Biological Bulletin, 183*, 233-254.

Gunamalai, V., & Subramoniam, T. (2002). Synchronisation of molting and oogenic cycles in a continuously breeding population of the sand crab *Emerita asiatica* on the Madras Coast, South India. *Journal of Crustacean Biology, 22*, 398-410.

Hinsch, G. W. & Cone, V. M. (1969). Ultrastructural observations of vitellogenesis in the spider crab, *Libinia emarginata* L. *Journal of Cell Biology, 40*, 336-342.

Komm, B. S., & Hinsch, G. W. (1987). Oogenesis in the terrestrial hermit crab, *Coenobita clypeatus* (Decapoda, Anomura): II. Vitellogenesis. *Journal of Morphology, 192*, 269-277.

Krol, R. M., Hawkins, W. E. & Overstreet, R. M. (1992). Reproductive components. In Harrison F. W. & Humes A. G. (Eds.), *Microscopic Anatomy of Invertebrates: Decapod Crustacea* (pp. 259-343). Hoboken, NJ: Wiley-Liss.

Kulkarni, G. K., Glade, L., & Finguerman, M. (1991). Oogenesis and effects of neuroendocrine tissues on in vitro synthesis of protein by the ovary of the red swamp crayfish *Procambarus clarkii* (Girard). *Journal of Crustacean Biology, 11*, 513-521.

Lee, C. Y., & Watson, D. (1995). In vitro study of vitellogenesis in the blue crab (*Callinectes sapidus*): site and control of vitellin synthesis. *Journal of Experimental Zoology, 271*, 364-372.

Lee, B. Y., Ng, N. K., & Ng, P. K. (2015). The taxonomy of five species of *Episesarma* De Man 1895, in Singapore (Crustacea: Decapoda: Brachyura: Sesarmidae). *Raffles Bulletin of Zoology, 31*, 199-215.

Mclay, C. L., & Becker, B. (2015). Reproduction in Brachyura. In Castro *et al.* (Eds.). *The Crustacea* (pp 185-243). Leiden, the Netherlands: Brill.

Meeratana, P., & Sobhon, P. (2007). Classification of differentiating oocytes during ovarian cycle in the giant freshwater prawn, *Macrobrachium rosenbergii* de man. *Aquaculture, 270*, 249-258.

Mota, M. I., & Tomé, G. S. (1965). On the histological structure of the gonads of *Panulirus argus* (Latr.). *Arquivos da Estação de Biologia Marinha da Universidade do Ceará, 5*, 15-26.

Moyano, M. P. S., Gavio, M. A., & Cuartas, E. I. (2010). Morphology and function of the reproductive tract of the spider crab *Libinia spinosa* (Crustacea, Brachyura, Majoidea): Pattern of sperm storage. *Helgoland Marine Research, 64*, 213-221.

Nieves, P. M., Olfindo, N. R., & Macale, A. M. (2015). Reproductive biology of Christian crabs (*Charybdis feriatus*, Linnaeus, 1758) in San Miguel Bay, Philippines. *Kuroshio Science, 9*(1), 13-16.

Okumura, T., Kim, Y. K., Kawazoe, I., Yamano, K., Tsutsui, N., & Aida, K. (2006). Expression of vitellogenin and cortical rod proteins during induced ovarian development by eyestalk ablation in the kuruma prawn, *Marsupenaeus japonicus*. *Comparative Biochemistry and Physiology Part A, 143*, 246-253.

Ongvarrasopone, C., Roshorm, Y., Somyong, S., Pothiratana, C., Petchdee, S., Tangkhabuanbutra, J., . . . Panyim, S. (2006). Molecular cloning and functional expression of the *Penaeus monodon* 5-HT receptor. *Biochimica et Biophysica Acta, 1759*, 328-339.

Presnell, J. K., & Schreibman, M. P. (1997). *Humason's animal tissue techniques* (5th ed.). Baltimore, MD: Johns Hopkins University Press.

Ravi, R., Manisseri, M. K., & Sanil, N. K. (2013). Ovarian maturation and oogenesis in the blue swimmer crab, *Portunus pelagicus* (Decapoda: Portunidae). *Acta Zoologica, 94*, 291-299.

Riley, H. G., & Tsukimura, B. (1998). Yolk protein synthesis in rice land tadpole shrimp, *Triops longicaudatus*, measured by in vitro incorporation of H3-leucine. *Journal Experimental Zoology, 281*, 238-247.

Sharifian, S., Kamrani, E., Safaie, M., & Sharifan, S. (2015). Oogenesis and ovarian development in the freshwater Crab *Sodhiana iranica* (Decapoda: Gecarcinuaidae) from the south of Iran. *Tissue and Cell, 47*, 213-220.

Stewart, M. J., Soonklang, N., Stewart, P., Hanna, P. J., Wanichanon, C., Parratt, A., . . . Sobhon, P. (2007). Histological studies of the ovaries of two tropical portunid crabs, *Portunus pelagicus* (L.) and *Scylla serrata* (F.). *Invertebrate Reproduction and Development, 50*, 85-97.

Subramoniam, T. (2017). *Sexual biology and-reproduction in crustaceans.* New York, NY: Academic Press.

Sudtongkong, C., Decharun, T. & Watttanagul, W. (2014). Survival rate of sesarmid crab larval *Episesarma singaporense* (Tweedie, 1936) fed with different proportions of formulated diet and Artemia. *Proceeding in Marine Science Conference*. 10-12 June 2014.

Sumpton, W. D., Potter, M. A., & Smith, G. S. (1994). Reproduction and growth of the commercial sand crab, *Portunus pelagicus* (L.) in Moreton Bay, Queensland. *Asian Fisheries Science, 7*, 103-113.

Suvarna, K. S., Layton, C., & Bancroft, J. D. (2013). *Bancroft's theory and practice of histological techniques* (7th ed.). Ontario, Canada: Elsevier.

Tantikitti, C., Kaonoona, R., & Pongmaneerat, J. (2015). Fatty acid profiles and carotenoids accumulation in hepatopancreas and ovary of wild female mud crab (Scylla paramamosain, Estampador, 1949). *Songklanakarin Journal of Science and Technology*, *37*(6), 609-616.

Teinsongrusmee, B. (2009). *Sesarmid crab* [in Thai]. Bangkok, Thailand: Thailand Research Fund (TRF).

Walker, A., Ando, S., Smith, G., & Lee, R. (2006). The utilization of lipovitellin during blue crab (*Callinectes sapidus*) embryogenesis. *Comparative Biochemistry and Physiology*, *143*, 201-208.

Wilder, M., Okumura, T., & Tsutsui, N., (2010). Reproductive mechanisms in crustacea focusing on selected prawn species vitellogenin structure, processing and synthetic control. *Aqua-BioScience Monographs*, *3*, 73-110.

Wilson, J. M., Bunte, R. M., Carty, A. J., (2009). Evaluation of rapid cooling and tricaine methanesulfonate (MS222) as methods of euthanasia in zebrafish (Danio rerio). *Journal of the American Association for Laboratory Animal Science*, *48*, 785–787.

Wu, X., Chang, G., Cheng, Y., Zeng, C., Southgate, P. C., & Lu, J. (2010). Effects of dietary phospholipid and highly unsaturated fatty acid on the gonadal development, tissue proximate composition, lipid class and fatty acid composition of precocious Chinese mitten crab, *Eriocheir sinensis*. *Aquaculture Nutrition*, *16*, 25-36.

Zara, F. J., Gaeta, H. H., Costa, T. M., Toyama, M. H., & Caetano, F. H. (2013). The ovarian cycle histochemistry and its relationship with hepatopancreas weight in the blue crab *Callinectes danae* (Crustacea: Portunidae). *Acta Zoolologica*, *94*, 134-146.