

# Gonad maturation of giant freshwater prawn, *Macrobrachium rosenbergii* at the bay of Padas River, Beaufort, Sabah, Malaysia

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**ABSTRACT** Giant freshwater prawn, *Macrobrachium rosenbergii* is a commercial species that is popular especially in Asian countries such as Malaysia, Thailand, Indonesia and others. Currently, there is a decline in *M. rosenbergii* due to dependency on natural resources and intensive reduction of *M. rosenbergii*. Thus, this study provides new information on the gonad maturation of *M. rosenbergii* especially in Padas River Bay, Beaufort, Sabah, Malaysia. A total of 40 tails in *M. rosenbergii* (20 tails of male and 20 tails of female) were obtained from Kampung Inuman, Beaufort, Sabah. The gonads of *M. rosenbergii* were dissected out and the stages of gonad maturation were identified through the histological examination. The body length (BL), body weight (BW) and carapace length (CL) for male and female of *M. rosenbergii* were  $16.90 \pm 2.19$  cm,  $50.55 \pm 47.83$  g,  $8.14 \pm 1.84$  cm,  $14.97 \pm 2.22$  cm,  $27.14 \pm 23.04$  g and  $6.55 \pm 1.68$  cm. There were five gonad maturation stages recognized comprised of 40% male and 30% female categorized in immature stage (stage I and II) while 60% male and 70% female were in matured stage (stage III, IV and V). In addition, gonadosomatic index (GSI) was not significant for male, while it was significant for female. In conclusion, five maturation stages were found at the Bay of Padas River Beaufort, Sabah and the giant freshwater prawn can reproduce more than once in their life cycle as well as their reproduction occurs throughout the year.

**KEYWORDS:** Gonad maturation, *Macrobrachium rosenbergii*, maturation stages, gonad weight, Gonadosomatic Index (GSI)

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## INTRODUCTION

The giant freshwater prawn (*Macrobrachium rosenbergii*) is a species of freshwater prawn known as "udang galah" in Malaysia. *M. rosenbergii* has a wide distribution in Indo-Pacific areas, including East Pakistan, India, Ceylon, Burma, Thailand, Malaysia, Indonesia, Philippines, Cambodia and Vietnam (Banu & Christianus, 2016). In addition, to meet the demand for the seed quality supply and continuousness, the quality of broodstock in *M. rosenbergii* was necessary (Sutarjo et al., 2021). Currently, the aquaculture of *M. rosenbergii* faced many problems, comprising diseases and stress during captivity that result in lower fecundity and reproduction (Kankuan et al., 2017). Furthermore, there have some attempts to increase the reproduction of *M. rosenbergii* by inducing the gonad maturation, reducing the gonad development period and spawning via special formula feed (Takács-Vellai et al., 2005; Ribeiro et al., 2012) or through hormone injections (Tinikul et al., 2009; Sumpownon et al., 2015; Thongbuakaew et al., 2016) or thru eye-stalk ablation (Okumura & Aida, 2001).

Gonad maturity is an important aspect of reproductive life history. The maturity stage of gonad at first maturity can be attributed to the measurement of fish or prawn. This information can serve as a basis for regulating the use of catching tools used to capture shrimp to reduce excessive exploitation pressures (Isnawati, 2004). The determination in the maturity stage of gonad was to

find out the comparison between mature and immature gonads for *M. rosenbergii*. The histological examination in gonad could be used to distinguish between mature and immature stage of ovary and testis in *M. rosenbergii*. Ovarian development stages of giant freshwater prawns were identified on the basis of their external appearance (color, shape and size), histological structure and the relative frequency of differentiating germ cells (Martins et al., 2007; Meeratana & Sobhon, 2007; Kai-Hsiang et al. (2010). This study was provided with an overview of biological reproduction, especially the gonad maturation of the male and female prawns of different sizes. Many studies on crustacean biological reproduction have been performed specifically on species that have high commercial or ecological potential (Soundarapandian et al., 2013). Studies on gonads, size attaining maturity and fertility are important aspects of biological reproduction for crustacean species. Knowledge of gonad maturation stages is also one of the biological information that can be used to predict the exact time for prawn to release their eggs (Isnawati, 2004). However, the knowledge on reproduction, especially gonad maturation is important and is the basic data for the management of sustainable prawn resources (Muhd-Farouk et al., 2014).

The Padas River is one of the rivers in Sabah which has become a source for prawn capture as an income for fishermen. Gonad maturity examination is basic knowledge in reproduction meanwhile further studies need to increase the production of *M. rosenbergii* especially for intensive cultivation and stock enhancement to balance the natural population of the area. The objective of the present study is to provide new information on the gonad maturation of *M. rosenbergii* especially at Padas River Bay, Beaufort, Sabah, Malaysia.

## MATERIALS AND METHODS

### *Study Area*

All the measurement of the present study was carried out at Biotechnology Lab, University Malaysia Sabah (UMS) from June until December 2019.

### *Sampling and Measurement of Different Traits*

*M. rosenbergii* were obtained and bought from Kampung Inuman, Weston, Beaufort, Sabah. A total of 20 male and 20 females (*M. rosenbergii*) were randomly selected and obtained for three months collections depending on the availability of supplies in July, August and September 2019. All samples were weighted and carapace length (CL) was measured (in centimeter). Once the measurement was done and the dissection was performed immediately to ensure the gonad is still fresh and not damaged. Measurement and dissection were performed at Borneo Marine Research Institute (BMRI) Biotechnology Lab, University Malaysia Sabah (UMS) to determine the gonad maturity of *M. rosenbergii* and the data were recorded.

### *Gonad Intake and Histological of Gonad*

After measuring the body length (BL), body weight (BW) and CL, the gonad of *M. rosenbergii* for each sample were dissected out. In addition, the reproductive organs of *M. rosenbergii* were observed to identify which one was male and female. The male has petasma while the female has thelycum. Using forceps, *M. rosenbergii* gonad was extracted slowly for histological purposes. This procedure was done slowly and carefully to avoid damaging the gonad. At the same time, the prawn gonad was weighed and fixed in bouin solution. For the histological analysis of gonad, the ovary and testis samples were immersed in bouin solution for less than 24 hours. For the fixation, the samples were dehydrated through a series of ethanol concentrations (80%, 90%, 95% and 100%) for every 30 minutes. The specimens were cleared using xylene two times. The samples were

immersed in paraffin two times for 60 min and embedded using the cube mould. The wooden block was used to block the samples. Microtome was used for sectioning the samples with the cutting thickness are 5 $\mu$ m (Fatimah et al., 2019). The specimens were stained passing through a series of xylene, then with decreasing ethanol concentration (70%, 95% and 100%), haemotoxylin and eosin Y. The samples were mounted with DPX or fixed with Canada balsam, then covered with glass. The specimen was observed under a compound microscope with a 40x magnificent scale.

#### Data Analysis

Statistical analyzes used were correlation and one-way ANOVA, ( $p < 0.05$ ) using SPSS software version 20.0. Applying the correlations to show the relationship between the size and BW followed by a Pearson t-test to show the significance between size and BW of *M. rosenbergii* for both sexes. One-way Variance analysis was used to show the comparisons between BW, CL and also gonadosomatic index (GSI) with maturity levels for both sexes. In addition, GSI was calculated and the percentages of gonad maturity (PGM) for both sexes were calculated. GSI was determined based on Equation (1).

$$\text{Gonadosomatic Index, GSI} = (\text{gonad weight} / \text{total tissue weight}) \times 100 \quad (1)$$

## RESULTS AND DISCUSSION

#### Gonad maturation in Male and Female of *Macrobrachium rosenbergii*

Measurements of BL, BW, CL, gonad weight (GW) and gonad maturity index for both genders are shown in Table 1. From the results, five stages of maturity for both males and female were identified (Figure 1 and Figure 2). In Figure 1a-d, there were the stages of testis maturation (male) and in Figure 2, there were five stages of ovarian maturation (female) in *M. rosenbergii*. The BL of small and large sized of *M. rosenbergii* were  $15.93 \pm 1.10$  cm and  $17.95 \pm 2.55$  cm for males, while there were  $13.85 \pm 1.42$  cm and  $16.08 \pm 2.38$  cm for females (Table 2). For stage I and II, there were eight males and six females while, for the stages III, IV and V, there were 12 males and 14 females (Table 3). Gonad maturity for males and females in stages I and II (immature) were 40% (mean size; 16.43 cm) and 30% (mean size; 12.06 cm). While, the PGM for male and female in stage III, IV and V (mature) were 60% (mean size is 16.43 cm) and 70% (mean size is 14.87 cm) (Table 3).

**Table 1.** Measurements (mean  $\pm$  standard deviation) for males and females in giant freshwater prawn, *Macrobrachium rosenbergii*

Measurement	Mean $\pm$ standard deviation	
	Male	Female
Body length (cm)	16.90 $\pm$ 2.19	14.97 $\pm$ 2.22
Body weight (g)	50.55 $\pm$ 47.83	27.14 $\pm$ 23.04
Carapace length (cm)	8.14 $\pm$ 1.84	6.55 $\pm$ 1.68
Gonad weight (g)	0.17 $\pm$ 0.22	0.98 $\pm$ 1.11
Gonadosomatic index (GSI)	0.30 $\pm$ 0.15	3.63 $\pm$ 2.78

**Table 2.** Body length in small and large sized for giant freshwater prawn, *Macrobrachium rosenbergii* that has reached maturity (body length  $\pm$  standard deviation)

	Body length $\pm$ standard deviation (cm)	
	Male	Female
Small	15.93 $\pm$ 1.10	13.85 $\pm$ 1.42
Large	17.95 $\pm$ 2.55	16.08 $\pm$ 2.38

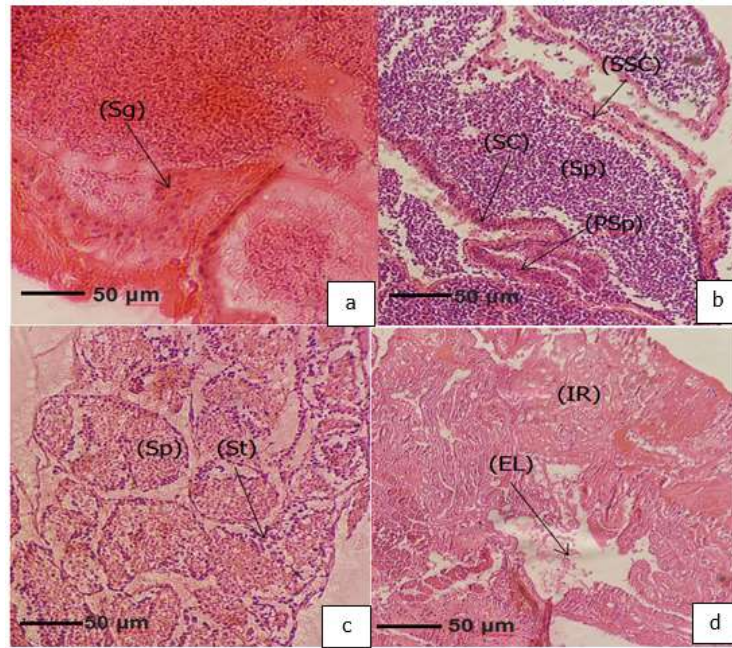
**Table 3.** Gonad maturity (%) and body length (mean  $\pm$  standard deviation) for males and females of giant freshwater prawn, *Macrobrachium rosenbergii*

Stage	Number		Mean (cm)		Gonad maturity (%)	
	Male	Female	Male	Female	Male	Female
I	2	2	16.43 $\pm$ 3.13	12.06 $\pm$ 1.82	Immature (40)	Immature (30)
II	6	4				
III	5	2				
IV	6	3	16.43 $\pm$ 3.44	14.87 $\pm$ 4.27	Mature (60)	Mature (70)
V	1	9				

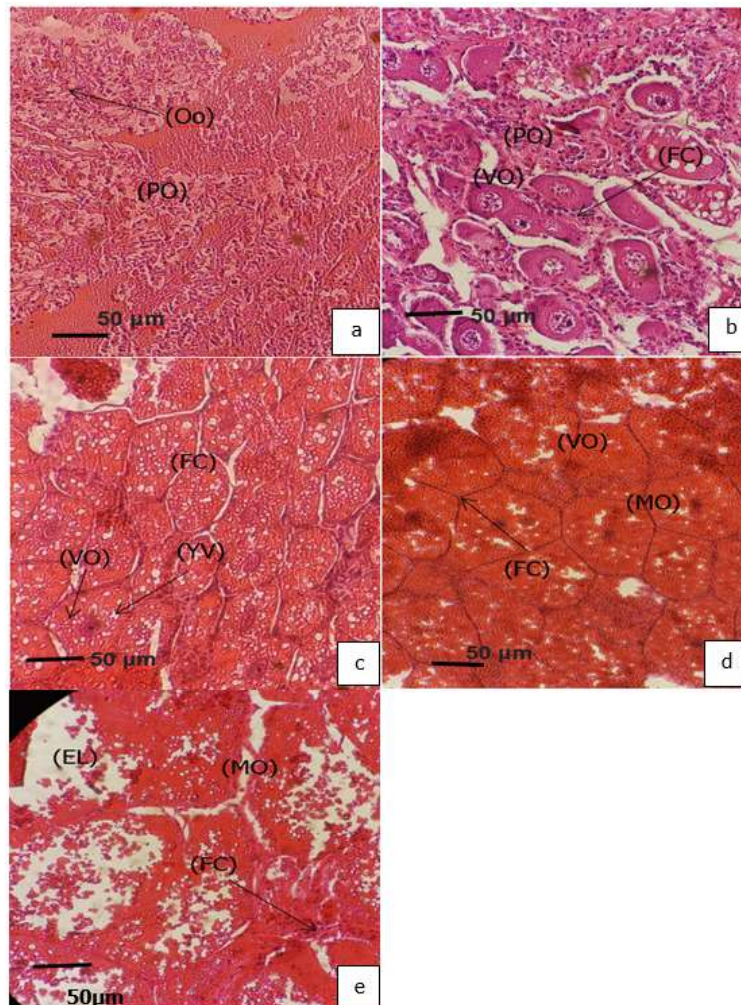
In this study, a total of 40 tails in *M. rosenbergii* were identify the gonad maturation for all stages in males and females during three months (July to September 2019). The mature of *M. rosenbergii* were higher than the immature for both sexes at Padas River Bay, Beaufort, Sabah, Malaysia. *M. rosenbergii* that has an annual breeding season had a different percentage of gonad maturity during each sampling period (Isnawati, 2004). The maturity level of *M. rosenbergii* gonad was one of important as a guide to predicting the peak season. Prawns have reached maturity especially stage V are ready to release eggs. After fertilization within 19-21 days, the eggs were hatched (Isnawati, 2004). According to Niamaimandi et al. 2008, excessive captivity of crustaceans led to changes in size to reach maturity. This also indicates that *M. rosenbergii* populations in the Padas River, Beaufort, Sabah was decreasing as the size of the mature prawn has been identified. Breeding usually occurs when the rainy season is in the tropics and the four-season, it usually occurs in the summer (Jadhav, 2008). However, spawning usually occurs in the rainy season but there are peaks in the particular month and depending on the area. Breeding or spawning peaks are different in each region (Khair et al., 2000). In Bagerhat, Khulna occurs from January to July, in the Hoogly Bay occurs from December to July and in the Cochin brackish areas from July to December (Khair et al., 2000). In the present study, the peaks of maturity and breeding were crucial in the management of prawn resources especially in the Padas River, Beaufort, Sabah. If breeding peaks occur from July to September, the area should be categorized as a restricted area for any activity that involves catching to allow prawns to breed without interruption. In addition, illegal *M. rosenbergii* captivity such as the use of chemical poisons must be enforced and laws must be enforced not only in the Padas River but also throughout the area as a source of capture and income to ensure the sustainability of these prawn sources.

In the natural environment, usually, the gonad maturation in *M. rosenbergii* was affected by these factors, which are food availability, temperature, current and suitable breeding grounds (Isnawati, 2004). According to Isnawati (2004), internal factors influence the maturity of the gonad such as hormone balance. Hormone balance is also closely linked to the source of food; if the food source is sufficient to support the survival of *M. rosenbergii* then a balance of hormones is also achieved. This is because hormones are a type of protein that is produced by the body through foods that are derived primarily from protein sources.





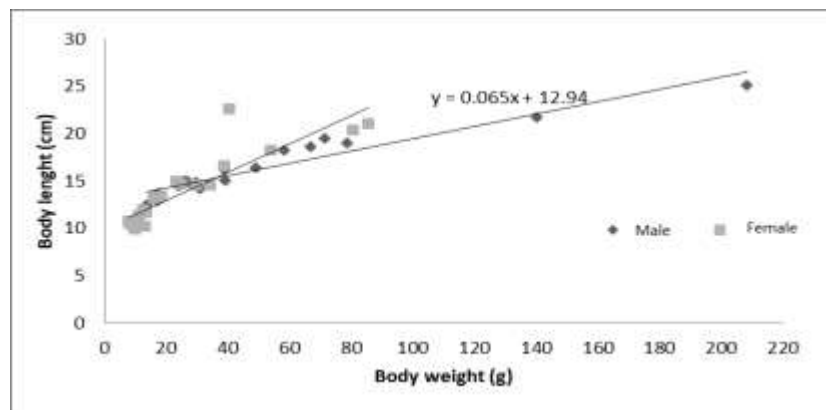
**Figure 1.** Stages of testis maturation based on histological observation. a) Stage I; b) Stage II & III; c) Stage IV; and d) Stage V. Sg - Spermatogium; SC - Spermatocytes; PSp - Primary spermatocytes; SSC - Secondary spermatocytes; St - Spermatid; Sp - Spermatozoa; EL - Empty lumen; IR - wrinkled and irregular shape



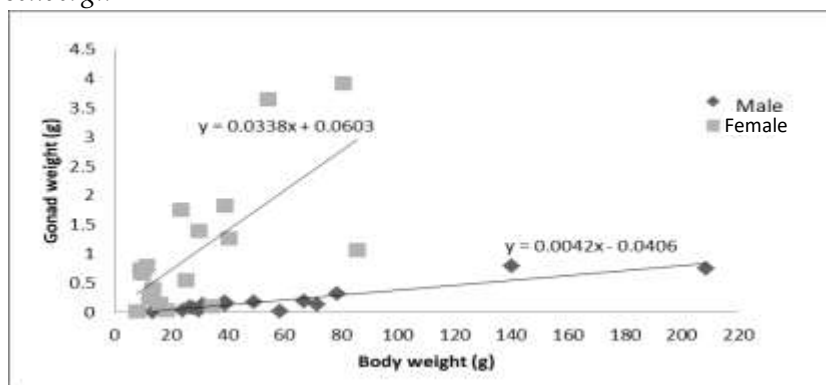
**Figure 2.** Five stages of ovarian maturation based on histological observations. a) Stage I; b) Stage II; c) Stage III; d) Stage IV; and E) Stage V. Oo - Oogonium; Po - Primary oocyte; VO - Vitellogensis oocyte; FC - Cells surrounding the follicle; YV - Vesicle yolk; Mo - Mature oocyte; EL - Empty lumen

### Relationship Between Body Length (BL) and Body Weight (BW), and Gonad Weight (GW) and Body Weight (BW)

The correlation,  $r = 0.956$  shows that BL and BW has a positive relationship (Figure 3). Therefore, the BL and BW relationship is significant for both sexes. There was a significant relationship between the GW and BW for both sexes (Figure 4). According to Huang et al. (2010), the maturity of freshwater crustacean gonads was influenced by their environmental parameters in natural habitats. BL and BW have a significant relationship in *M. rosenbergii*. A study by Kunda et al. (2008) showed BL has a significant positive relationship in BW of *M. rosenbergii*. BL and BW relationships were important in fisheries. It helps to establish the relationship between the two variables and enables the conversion of one variable into another variable, as well as to explain species growth and to determine the differences in different stocks for the same species (Lalrinsanga et al., 2012).



**Figure 3.** Relationship between body length and body weight in giant freshwater prawn, *Macrobrachium rosenbergii*



**Figure 4.** Relationship between gonad weight and body weight in giant freshwater prawn, *Macrobrachium rosenbergii*

### Relationship Between Body Weight (BW) and Gonadosomatic Index (GSI) With Gonad Maturity

Based on Table 4, at the 95% of confidence level for the relationship between BW and GSI with gonad maturity, there is  $P > 0.05$  for males and  $P > 0.05$  in BW whereas for female,  $P < 0.05$  in GSI. According to Chang & Shih (2011), the maturity stage in female gonad is closely related to GSI, where the increase in GSI is also influenced by the increase in maturity stage due to an increase in the gonad volume after reaching the mature stage. For male testicular, weight gain was not significant while weight gain for females was significant by increasing the maturity stage. Likewise, BW or BL does not affect the maturity in the gonad of *M. rosenbergii*. It can be concluded that only GSI female *M. rosenbergii* was used as a guide to predict gonad maturity and not suitable only state the BW and BW to identify the stages of gonad maturity in *M. rosenbergii*.

**Table 4.** One-way ANOVA at the 95% confidence level for body weight and GSI with gonad maturity

One-way ANOVA	Weight Confidence Level for 95%	
	Male	Female
Weight with gonad maturity	0.326 > 0.05	0.46 > 0.05
GSI with gonad maturity	0.951 > 0.05	0.00 < 0.05

## CONCLUSION

All the maturity stages in *M. rosenbergii* were identified for both sexes in the present study. The percentage of *M. rosenbergii* was reached at a high level of maturity giving a positive indication of the peak of maturity occurring during the month. However, due to constraints during the study period, the number of samples were quite limited and not sufficient enough for an in-depth study of the maturity stage of the lobster gonad especially in Padas River Bay, Beaufort, Sabah to represent the entire population. For future perspective, deeper analysis with larger samples should be carried alongside with breeding studies on gonad maturity.

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## REFERENCES

- [1] Banu, R. & Christianus, A. 2016. Giant freshwater prawn *Macrobrachium rosenbergii* Farming: A review on its current status and prospective in Malaysia. *Journal of Aquaculture Research and Development*, 7(4), 1-5.
- [2] Chang, C. F. & Shih, T. W. 2011. Reproductive cycle of ovarian development and vitellogenin profiles in the freshwater prawns, *Macrobrachium rosenbergii*. *Invertebrates Reproduction and Development*, 1(27), 11-20.
- [3] Fatimah, S. N., Halik, N. A. S. A. & Muhd-Farouk, H. 2019. Determine the sperm quantity and histological characteristics on the testes of male mud crab, *Scylla tranquebarica*. *Asian Journal of Applied Sciences*, 12(4), 173-179.
- [4] Huang, K. H., Wu, J. P., Wang, S. Y., Huang, D. J. & Chen, H. C. 2010. Ovarian development in the freshwater prawn, *Macrobrachium asperulum* (Decapoda: Palaemonidae). *Journal of Crustacean Biology*, 30(4), 615-623.
- [5] Isnawati, M. 2004. Kajian tingkat kematangan gonad udang galah (*Macrobrachium rosenbergii* de Man) di Muara Sungai Kapuas Pontianak Kalimantan Barat. MSc Thesis, Institut Pertanian Bogor.
- [6] Jadhav, U. 2008. *Aquaculture Technology and Environment*. New Delhi: PHI Learning.
- [7] Kai-Hsiang, H., Jui-Pin, W., Shu-Yin, W., Da-Ji, H. & Hon-Cheng, C. 2010. Ovarian development in the prawn *Macrobrachium asperulum* (Decapoda: Palaemonidae). *Journal of Crustacean Biology*, 30(4), 615-623.
- [8] Kankuan, W., Wanichanon, C., Titone, R., Engsusophon, A., Sumpownon, C., Suphamungmee, W., Morani, F., Masini, M., Novelli, M., Isidoro, C. & Sobhon, P. 2017. Starvation Promotes Autophagy-Associated Maturation of the Ovary in the Giant Freshwater Prawn, *Macrobrachium rosenbergii*. *Frontiers in Physiology*, 8(300), 1-12.
- [9] Khair, S. A., Hossain, M. A. & Parween, S. 2000. Distribution of *Macrobrachium rosenbergii* (de Man) in three rivers of Paikgacha, Bangladesh. *Bangladesh Journal of Fisheries Research*, 4(2), 199-202.



- [10] Kunda, M., Dewan, S., Uddin, M. J., Karim, M., Kabir, S. & Uddin, M. S. 2008. Short communication: Length-weight relationship, condition factor and relative condition factor of *Macrobrachium rosenbergii* in rice fields. *Asian Fisheries Science*, 21, 451-456.
- [11] Lalrinsanga, P. L., Pillai, B. R., Patra, G., Mohanty, S., Naik, N. K. & Sahu, S. 2012. Length weight relationship and condition factor of giant freshwater prawn, *Macrobrachium rosenbergii* (De Man, 1879) based on developmental stages, culture stages and sex. *Turkish Journal of Fisheries and Aquatic Sciences*, 12, 917-924.
- [12] Martins, J., Ribeiro, K., Rangel-Figueiredo, T. & Coimbra, J. 2007. Reproductive cycle, ovarian development, and vertebrate-type steroids profile in the freshwater prawn *Macrobrachium rosenbergii*. *Journal of Crustacean Biology*, 27, 220-228.
- [13] 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.
- [14] Muhd-Farouk, H., Abol-Munafi, A. B., Jasmani, S. & Ikhwanuddin, M. 2014. Effect of steroid hormones 17 $\alpha$ -hydroxyprogesterone and 17 $\alpha$ -hydroxypregnenolone on ovary external morphology of orange mud crab, *Scylla olivacea*. *Asian Journal of Cell Biology*, 9, 23-28.
- [15] Niamaimandi, N., Aziz, A., Khalijah, D. S., Roos, S. C. & Kiabi, B. 2008. Reproductive biology of the green tiger prawn (*Penaeus semisulcatus*) in coastal waters of Bushehr, Persian Gulf. *ICES Journal of Marine Science*, 65(9), 1593–1599.
- [16] Okumura, T. & Aida, K. 2001. Effects of bilateral eyestalk ablation on molting and ovarian development in the giant freshwater prawn, *Macrobrachium rosenbergii*. *Fisheries Science*, 67, 1125–1135.
- [17] Ribeiro, K., Franceschini-Vicentini, I. B., Papa, L. P., New, M. B. & Valenti, W. C. 2012. Effect of polyunsaturated fatty acids on the fecundity of the Amazon River prawn *Macrobrachium amazonicum* (Heller, 1862). *Aquaculture Research*, 43, 1756–1763.
- [18] Soundarapandian, P., Varadharajan, D. & Boopathi, A. 2013. Reproductive biology of the commercially important Portunid crab, *Portunus sanguinolentus* (Herbst). *Marine science Research and Development*, 3(2), 1-9.
- [19] Sumpownon, C., Engsusophon, A., Siangcham, T., Sugiyama, E., Soonklang, N., Meeratana, P., Wanichanon, C., Hanna, P. J., Setou, M. & Sobhon, P. 2015. Variation of prostaglandin E2 concentrations in ovaries and its effects on ovarian maturation and oocyte proliferation in the giant fresh water prawn, *Macrobrachium rosenbergii*. *General and Comparative Endocrinology*, 223, 129–138.
- [20] Sutarjo, G. A., Hermawan, D. & Nisa, A. Z. 2021. Effect of different of feed (squid, sea worms, and trash fish) to gonad maternity and fundamental levels of Galah shrimp (*Macrobrahium rosenbergii*). *Indonesian Journal of Tropical Aquatic*, 4(1), 24-30.
- [21] Takács-Vellai, K., Vellai, T., Puoti, A., Passannante, M., Wicky, C., Streit, A., Kovacs, A. L. & Muller, F. 2005. Inactivation of the autophagy gene bec-1 triggers apoptotic cell death in *C. elegans*. *Current Biology*, 15, 1513–1517.
- [22] Thongbuakaew, T., Saetan, J., Suwansa-ard, S., Kankoun, W., Sumpownon, C., Parhar, I., Meeratana, P., Sobhon, P. & Sretarugsa, P. 2016. The existence of kisspeptin-like peptides and effects on ovarian development and maturation in the giant freshwater prawn, *Macrobrachium rosenbergii*. *Journal of Aquaculture Research and Development*, 455, 50–60.
- [23] Tinikul, Y., Soonthornsumrith, B., Phoungpetchara, I., Meeratana, P., Poljaroen, J., Duangsuwan, P., Soonklang, N., Mercier, A. J. & Sobhon, P. (2009). Effects of Serotonin, Dopamine, Octopamine, and Spiperone on Ovarian Maturation and Embryonic development in the giant freshwater prawn, *Macrobrachium Rosenbergi* (De Man, 1879). *Crustaceana*, 82(8), 1007–1022.