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Short Communication

Evaluating Ovarian Maturation Stages of Banana Shrimp, *Penaeus merguiensis*

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ABSTRACT

The present study investigated the ovarian development of female banana shrimp, *Penaeus merguiensis* (n = 50), with a focus on external morphological and histological assessments. The findings indicated that *P. merguiensis* undergoes five distinct ovarian developmental stages: immature, premature, mature, fully mature, and spent. Externally, the ovaries changed in colour from transparent to whitish-yellow, light green, dark green, and finally white again from the immature to spent stages. Histologically, the presence of perinucleolar and yolkless oocytes characterised the immature and premature stages, whereas yolky oocytes predominated during the mature and fully mature stages. The gonadosomatic index (GSI) and oocyte size increased significantly from the immature to fully mature stages before decreasing significantly at the spent stage ($p < 0.05$). Understanding these ovarian developmental stages may contribute to the domestication and reproductive management of this species, particularly for broodstock maturation and seed production.

Keywords: Banana shrimp, ovarian maturation

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INTRODUCTION

Penaeus merguiensis is reported to be one of the most economically important shrimp species and a key catch in Malaysia (Hurzaid et al., 2023). Commonly known as the banana shrimp, *P. merguiensis* is considered a promising candidate for aquaculture in Malaysia due to its firm texture and fine flavour. This species is abundantly found in coastal areas and local markets across Malaysia, particularly in the northern regions of Peninsular Malaysia (Kedah, Perak, and Pulau Pinang), the southern region (Selangor and Johor), the eastern coast (Kelantan and Terengganu), as well as in East Malaysia (Sarawak), where it is primarily harvested from the wild by local fishermen (Abdul Halim et al., 2023; Aziz et al., 2020). *P. merguiensis* offers several advantages for shrimp farmers, including its large size (Robalino et al., 2016), rapid growth rate (Vance & Rothlisberg, 2020), and resistance to diseases (Wiradana et al., 2020). Hence, *P. merguiensis* appears to be a suitable candidate for aquaculture due to its wide availability of broodstock in Malaysia and its favourable biological traits. The culture of native species with abundant broodstock resources may support small-scale farmers in rural areas in establishing sustainable shrimp farming operations.

However, there is limited information on the basic reproductive biology of female *P. merguiensis*, which is critical for their use as broodstock in aquaculture. Although reproductive studies have been conducted in several penaeid shrimp species, information on ovarian maturation stages in female *P. merguiensis*, including external morphology, oocyte development, histological characteristics, and gonadosomatic index (GSI), remains limited. Typically, most penaeid shrimp undergo five distinct ovarian maturation stages, which can be detected through visual observation and confirmed more accurately through histological assessment (Manan et al., 2022). Previously, several penaeid species, including the Indian white prawn, *Penaeus indicus* (Amanat & Qureshi, 2011), giant freshwater prawn, *Macrobrachium rosenbergii* (Revathi et al., 2012), and whiteleg shrimp, *Litopenaeus vannamei*, have been reported to exhibit five ovarian maturation stages characterised by distinct ovarian colour changes from translucent, yellow, light green and ending with dark green, accompanied by progressive increases in gonadosomatic index (GSI) and oocyte size with advancing maturation stages. Therefore, the present study aims to provide detailed information on the ovarian maturation of *P. merguiensis* through assessments of external morphology and histology, covering ovarian colour, GSI, oocyte diameter and yolk progression. Identification of ovarian developmental stages can help determine the spawning season and reproductive cycle of *P. merguiensis*, which is important for establishing appropriate fishing seasons, protecting spawning stocks, and supporting sustainable resource management. In hatchery and captive breeding programs, information on ovarian maturation can be used to accurately select mature broodstock for mating and spawning induction, thereby improving fertilisation success and larval production.

MATERIALS AND METHODS

Sample Collection

Healthy gravid female *P. merguiensis* specimens were collected from the estuarine area of Kuala Muara, Pulau Sayak, and conditioned in a holding tank at the Department of

Fisheries (DOF) Pulau Sayak hatchery prior to the experiment. A total of 50 female samples (mature females weighing 25-35 g with a total length greater than 14 cm) were collected from the sampling site during low tide between June and August 2025. During collection, water temperature, salinity and dissolved oxygen were 28-30 °C, 28-30 ppt, and 6 mg/L, respectively. Samples were then subjected to physical observation, followed by dissection and histological assessment. Mature female selection was based on the presence of visible ovaries located dorsally to the cephalothorax. When the ovaries were not clearly visible, females were identified by the presence of the thelycum located between the fourth and fifth pereopods (walking legs), which serves as the reproductive structure in female penaeid shrimp (Moraes et al., 2025). All females were subsequently placed in ice-filled containers to immobilise them before dissection. Dissection began by carefully removing the exoskeleton behind the carapace, proceeding toward the telson. All procedures involving animal handling were followed by research ethics guidelines from the Office of Research Management (RMO), Universiti Malaysia Terengganu (UMT), Kuala Terengganu, for the care and use of animals in this whole experiment as outlined in the Research Ethics Guidelines for the Ethics of Research on Animals 2014 (UMT; https://studylib.net/doc/8738550/research-ethics-guidelines—pusat-pengurusan-penyelidikan).

Gonadosomatic Index (GSI)

Body weight and ovary weight were measured and recorded to determine the gonadosomatic index (GSI). The GSI represents the relative size of the gonads in proportion to the total body weight. It was calculated using the following formula in Equation 1:

GSI: [ovary weight (g)/ total weight (g)] * 100 (Aaqillah-Amr et al., 2022) [1]

Histology

Ovarian tissues were immediately fixed in Davidson's fixative at a tissue-to-fixative ratio of 1:10 (w/v) for 24 h at room temperature, followed by dehydration in 70% ethanol overnight. The dehydrated samples subsequently underwent tissue processing through a graded ethanol series of 70%, 90%, 95%, and 100% ethanol for 2, 1, 1, and 4 h, respectively, followed by clearing in xylene for 6 hours. The samples were then infiltrated with paraffin wax at 60 °C for 4 hours prior to embedding. Embedded tissues were sectioned at a thickness of 5 µm using a rotary microtome. The sections were dried on a hot plate at 40 °C overnight and subsequently stained with hematoxylin and eosin (H&E) for 10 and 3 minutes, respectively, following the standard staining protocol described by Fischer et al. (2008).

Statistical Analysis

All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 26. Data for gonadosomatic index (GSI) and oocyte diameter were expressed as mean ± standard deviation (SD). Normality and homogeneity of variance were evaluated using the Shapiro-Wilk and Levene's tests, respectively. Differences in GSI and oocyte diameter among ovarian maturation stages were analysed using one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison test for pairwise comparisons when significant differences were detected. Statistical significance was accepted at $P < 0.05$.

RESULTS

External Morphology

Table 1 illustrates the external morphology of the ovarian development stages in *P. merguiensis*. Ovary colour progressed from translucent (Stage 1), yellowish (Stage 2), light green (Stage 3), green (Stage 4), to whitish (Stage 5). The gonadosomatic index (GSI) increased significantly from Stage 1 (6.32 ± 0.80), Stage 2 (10.96 ± 1.20), Stage 3 (13.32 ± 1.68), to Stage 4 (19.70 ± 1.27), before decreasing significantly at Stage 5 (7.22 ± 0.68), with $p < 0.05$.

Histological Observation

Table 1 and Figure 1 indicated the histology of the ovarian development stages in *P. merguiensis*. Table 1 showed mean oocyte diameter increased from Stage 1 (39.4 ± 9.42 µm), Stage 2 (76.19 ± 8.89 µm), Stage 3 (134.04 ± 15.89 µm) to Stage 4 (152.04 ± 13.1 µm), then decreased during the spent stage (48.34 ± 21.9 µm), with Figure 1 illustrated Stage 1 and Stage 5 were characterised by the presence of perinucleolar $p < 0.05$. oocytes, Stage 2 by the appearance of yolkless oocytes, Stages 3 and 4 by the presence of yolky oocytes, with Stage 4 additionally marked by the presence of elongated cortical bodies.

DISCUSSION

The five ovarian developmental stages identified in this study are consistent with those reported for the same species, *P. merguiensis,* by Ayub and Ahmed (2002). Manan et al. (2022) reported similar ovary stages from Stage 1 to Stage 4 but did not observe the spent stage. Similarly, other penaeid shrimp species such as the Indian white prawn, *P. indicus* (Amanat and Qureshi, 2011), redtail shrimp, *P. penicillatus*, jinga shrimp, *Metapenaeus affinis*, kiddi shrimp, *P. stylifera* (Ayub & Ahmed, 2002), giant freshwater prawn, *M. rosenbergii* (Revathi et al., 2012), and white-leg shrimp, *P. vannamei* (Li et al., 2022) have

Table 1
External morphology and histology of banana shrimp, P. merguiensis *ovaries*

Stage	External Morphology		Histology	
	Description	**GSI**	**Description**	**Oocyte size (mm)**
Stage 1 (Immature)	The ovary was difficult to observe physically. It appeared as a straight, thin line along the abdomen and was clear, transparent, or translucent in colour.	6.32 ± 0.80^{a}	Perinucleolar oocytes were abundantly present in the ovary	39.43 ± 9.42^{a}
Stage 2 (Premature)	A more developed ovary was observed. It appeared as parallel lobes along the abdomen and was yellowish-white in colour.	10.96 ± 1.20^{b}	Perinucleolar oocytes and yolkless oocytes were identified in the ovary, with an increase in nucleus size while reducing cytoplasm volume.	76.19 ± 8.89^{b}
Stage 3 (Mature)	The ovary occupied a significant portion of the abdominal cavity, including the stomach in the cephalothorax region. Its colour was light green	13.32 ± 1.68^{c}	Yolky oocytes were more abundant than yolkless oocytes. Late-stage yolk globules were identified, and the cytoplasm volume had increased.	134.04 ± 15.89^{c}
Stage 4 (Fully mature)	The ovary filled the entire abdomen and cephalothorax. Its colour was bright green, but slightly darker compared to the previous stage.	19.70 ± 1.27^{d}	Abundant yolky oocytes with elongated cortical rods and yolk globules were identified.	152.04 ± 13.08^{d}
Stage 5 (Spent)	The ovary size was shrinking but remained larger than in Stage 1. Its colour was pale cream or whitish.	7.22 ± 0.68^{a}	Perinucleolar oocytes were present in fair numbers, along with follicle cells and chromatin nucleolar oocytes. Atretic oocytes were also present, undergoing reabsorption	48.34 ± 21.85^{a}

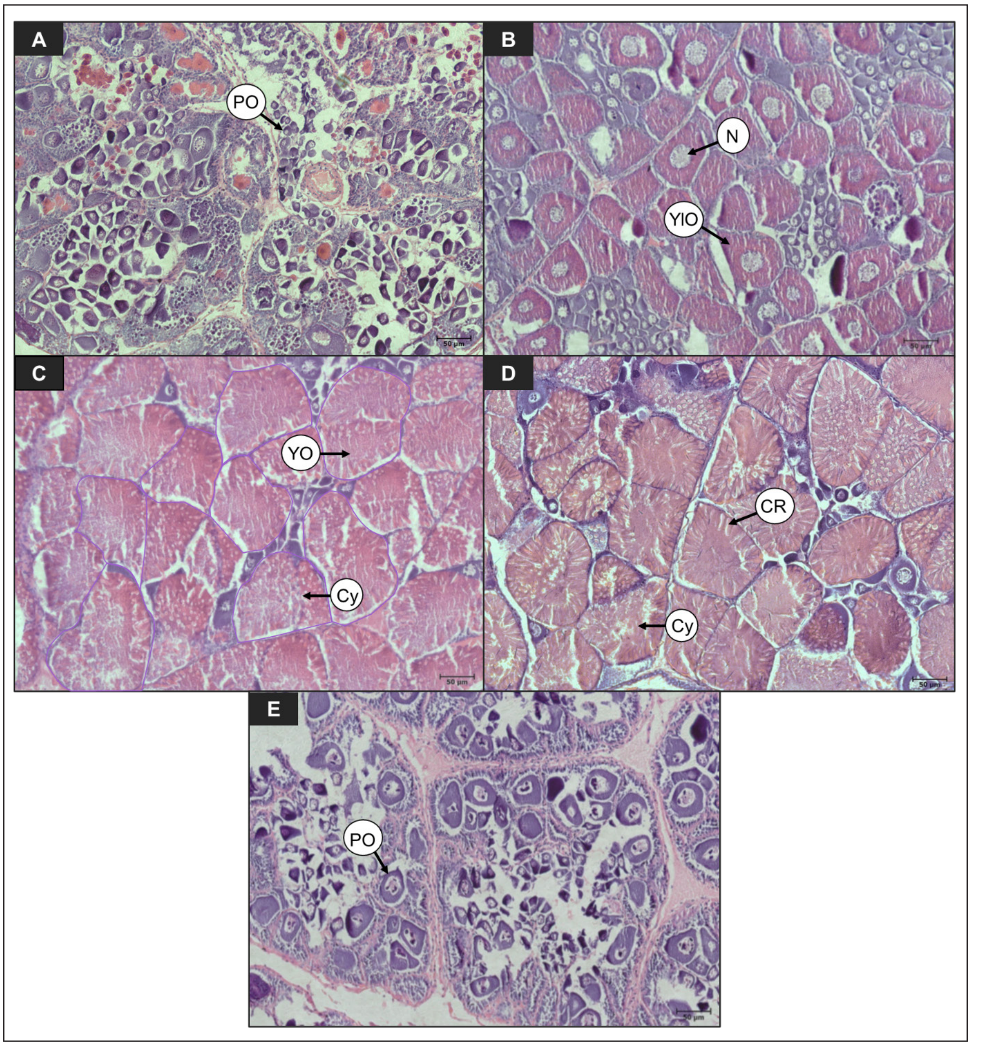

Figure 1. Ovary maturation stages of *P. merguiensis*: (A) immature, (B) premature, (C) mature, (D) fully mature, (E) spent stage. Histological features include (Cy) cytoplasm, (CR) cortical rod, (N) nucleus, (PO) perinucleolar oocytes, (YlO) yolkless oocytes, and (YO) yolk oocytes

also been classified into five ovarian stages. When compared to the present study, these species show a similar pattern of ovary colour progression, starting from translucent in early stages, turning yellowish, and reaching peak maturation with dark green colouration, followed by a whitish colour at the spent stage.

During the early ovarian development stage (Stage 1), perinucleolar oocytes dominate the ovary due to the lack of yolk. Yolk accumulation begins in Stage 2 as oocyte size increases; however, this stage is still dominated by yolkless oocytes with relatively large nuclei. In Stage 3, the nucleus was reduced and became faint, while yolky oocytes became more abundant than yolkless oocytes, resulting in an increase in oocyte size. In Stage 4, yolky oocytes dominated the entire ovary, with no visible nucleus and larger oocyte size than in Stage 3. Stage 4 was considered fully mature and represented the optimal nutrient supply for embryonic development in shrimp eggs. In stage 5, the GSI decreases because most mature oocytes and yolk reserves have already been released during spawning. All these processes are collectively referred to as vitellogenesis, which involves the accumulation of yolk within the ovary. The yolk consists of nutrients such as proteins and lipids, specifically vitellin, a type of lipoprotein (an apolipoprotein that binds lipids), which may be synthesised either in the ovary, the hepatopancreas, or both (Revathi et al., 2012). This mechanism is supported by Li et al., who reported the expression of several lipoprotein-related genes, including Vg, ApoD, BGBP, CP, VgR, VLDLR, and LDLR-RP1, in the hepatopancreatic and ovarian transcriptomes of white leg shrimp, *Litopenaeus vannamei* during ovarian maturation. The lipoproteins from the hepatopancreas are transported to the ovary via haemolymph, as shown by Revathi et al. (2012), who observed increased lipoprotein levels in the hemolymph with advancing ovarian stages in the giant freshwater prawn, *M. rosenbergii*. The vitellogenesis process enlarges oocyte size at the end of the ovarian stage. Similar histological evidence of vitellogenesis has also been reported in other crustacean species, including the mud crab, *Scylla olivacea* (Hidir et al., 2018), blue swimming crab, *Portunus pelagicus* (Ravi et al., 2012), giant freshwater prawn, *M. rosenbergii* (Revathi et al., 2012), white leg shrimp, *P. vannamei* (Quesada-Avila et al., 2024), and red swamp crayfish, *Procambarus clarkii* (Lu et al., 2020).

Vitellin is also responsible for the colour changes in the ovary, which becomes yellowish and green in the fully mature stage. Ovary colour is further influenced by esterified carotenoids, complexes formed between carotenoids and lipids, including β-carotene, zeaxanthin, and astaxanthin, which contribute to the pigmentation of the oocytes (Li et al., 2022). Additionally, cortical rods were clearly observed in Stage 4 ovaries, indicating the completion of oocyte maturation in penaeid shrimp. Cortical rods are glycoprotein-rich structures that develop at the final stage of vitellogenesis and are arranged around the periphery of mature oocytes (Kruevaisayawan et al., 2010). Upon spawning and fertilisation, these cortical materials are released through the cortical reaction, forming a jelly-like protective envelope surrounding the egg (Subramoniam, 2017). This envelope functions as a barrier that helps prevent polyspermy, microbial invasion, and environmental stress during early embryonic development, particularly in externally fertilised crustaceans such as penaeid shrimp (Subramoniam, 2017).

CONCLUSION

There are five stages of ovarian maturation in *P. merguiensis*: immature, premature, mature, fully mature, and spent. These stages are differentiated based on ovary colour, gonadosomatic index (GSI), and histological assessment, including the presence of perinucleolar oocytes, yolkless oocytes, yolky oocytes, and cortical rods. In the future, information regarding ovarian developmental stages in *P. merguiensis* may contribute to a better understanding of the spawning season and reproductive cycle of this species, which could support the establishment of appropriate fishing seasons, protection of spawning stocks, and sustainable fisheries management. Likewise, information on ovarian maturation may be further applied in hatchery and captive breeding programs for accurate broodstock selection, spawning induction, and improvement of fertilisation success and larval production.

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CONFLICT OF INTEREST

The authors declare no conflict of interest. The funders had no role in the design of the study, in the collection, analyses, or interpretation of data, in the writing of the manuscript, or in the decision to publish the results.

DATA AVAILABILITY STATEMENT

All data supporting the findings of this study are included within the paper. No additional data are available.

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