The Reproductive Cycle of the Female Blue Swimming Crab *Portunus pelagicus* (Linnaeus, 1758) from Kung Krabaen Bay, the Eastern Gulf of Thailand: Implications to Support Fisheries Management

Chutapa Kunsook¹ and Pongchai Dumrongrojwatthana²

1. Department of Biology, Faculty of Science and Technology, Rambhai Barni Rajabhat University, Chanthaburi Province 22000, Thailand
2. Department of Biology, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand

**Abstract:** The blue swimming crab, *Portunus pelagicus* (Linnaeus, 1758), is one of the most economically important crustaceans in Thailand. In order to gather more in-country data on its biological aspects, a comprehensive observation of the annual reproductive cycle of female *P. pelagicus*, which were gathered each month from Kung Krabaen Bay, Thailand, from October 2017 to September 2018 was undertaken. The ovarian structure, gonadosomatic index (GSI) and histological description of *P. pelagicus* were observed during this time. It was revealed that six distinct stages—I (immature stage), II (early maturing stage), III (late maturing stage), IV (early matured stage), V (middle matured stage) and VI (late matured stage)—occur during the annual reproductive cycle of the females. Moreover, the percentage of the oogenic proportion of *P. pelagicus* appeared continuously throughout the year. The ovarian development was exclusively found in stage VI, during October 2017 to December 2017, with the highest peak in November 2017 (39.96%) in relation to the high GSI. From October 2017 to February 2018, stage I was also observed, especially during October 2017 (49.5%), implying its important involvement in supporting oogenic production. It would be suggested that the successive spawning season of the *P. pelagicus* population clearly occurred during October-December each year. Therefore, this would be the best time to support hatchery seed production, during which *P. pelagicus* collection should be avoided.

**Key words:** Blue swimming crab, histology, oocyte, Thailand.

1. Introduction

Comprehensive information regarding the structural features of the reproductive systems and gonadal development of decapod crustaceans is proposed as both important and fundamental for ethological and reproductive cycle studies, which is in direct correlation with offspring survival for fisheries management, as well as sustainability for decapod populations [1-4]. The processes of oocyte growth and ovarian development in decapods during the reproductive cycle are complex mechanisms for cellular, morphological and colour changes that determine reproductive status and performance. Many historical reports concerning the economic importance of ovarian development for decapods have described the reproductive patterns as having great variation, determined through several comprehensive methods using seasonal fluctuations of the gonadosomatic index (GSI) [5], fecundity and egg batches [6], and ovarian histological changes [3]. According to Stewart *et al.* [3], there are four stages relating to varian morphology and gamete proportion: spawn-spent (stage I), proliferative (stage II), pre-mature (stage III) and mature (stage IV). Furthermore, the classification of the ovarian cycle in *Portunus pelagicus* was based
on histological structure and different cellular compositions. In terms of ovarian development, four stages, which include stages I through IV, were seen [7].

_P. pelagicus_ (Linnaeus, 1758), colloquially known as the “blue swimming crab”, is commonly used as a food resource of great commercial importance in Thailand, especially in Kung Krabaen Bay [8, 9]. With regards to Thai consumption, _P. pelagicus_ has an average yield of 43,871 tons per year. However, the declining production of this crab is noted at about 20,000 tons when compared to 2011 [10]. Unfortunately, these crabs are only collected in natural habitats, meaning that crab stocks can be easily overfished. Overcapacity and reducing the rate of loss of the crabs are considered to be a hot issue [8]. Due to declining production threatening the sustainability of blue swimming crab fisheries in natural habitats, as well as in Kung Krabaen Bay, the comprehensive information of _P. pelagicus_ has been continuously observed throughout the feeding ecology [9] in order to determine the population structure and to make stock assessments [11, 12]. Regrettably, an accurate reproductive cycle assessment of this crab is limited to scientific knowledge primarily based on histological observation. In this research, the natural reproductive cycle of _P. pelagicus_ females was investigated using a combination of existing comprehensive methods (GSI, external morphology and ovarian histological observation) in order to acquire data relating to their reproductive activities. These data would then subsequently aid the development of protection and conservation strategies for this crab within Kung Krabaen Bay, Thailand.

2. Materials and Methods

2.1 Crab Collections and Study Area

A total of 136 female blue swimming crabs, _P. pelagicus_, were caught monthly from Kung Krabaen Bay, Thailand (12°34'–12°12' N, 101°53’–101°55’ E), and analyzed throughout the study (October 2017 to September 2018). This area is a small, semi-enclosed estuarine system which is considered to be the largest zone for blue swimming crab fisheries in the country. The temperature of the area was also recorded during sample collections.

2.2 Ovarian Morphology and Gravimetric Analyses

In the laboratory, all _P. pelagicus_ were euthanised using a rapid cooling method for 30 min [13]. The body weight (BW) and carapace width (CW) were measured with calipers to the nearest 0.01 cm. In accordance with proper crab dissection, the dorsal part of the carapace was opened to observe the localisation of the female reproductive system. They were submerged in Ringer’s solution and photographs were taken using a digital camera (Cannon 650D) to observe their reproductive morphology. Lastly, the ovarian tissue of the berried females was dissected out and removed to study the GSI. The GSI was calculated using the following formula: GSI = gonadal weight/total weight × 100.

2.3 Histological Observations

In accordance with histological observation, ovarian tissues of the berried females were immediately suspended in Davidson’s fixative (330 mL 95% ethyl alcohol, 220 mL 100% formalin, 115 mL glacial acetic acid and 335 mL distilled H₂O [14] overnight at room temperature and subsequently processed using a standard histological technique [15, 16]. The paraffin blocks were cut at 4 µm of thickness and stained with Harris’s hematoxylin and eosin (H&E) and periodic acid Schiff (PAS) [15, 16]. The ovarian structure and stage of ovarian development of _P. pelagicus_ were determined by following criteria adopted from previous documents [8, 17] and taken with a Leica DM2000 light microscope (Boston Industries, Inc; USA).
3. Results and Discussion

3.1 Environmental Parameters

The mean value of the temperature was about 30.87 °C with range of 29.3-37.5 °C. The temperature during November to December 2017 was more varied, averaging at 29.55 °C.

3.2 CW and GSI

A total of 136 *P. pelagicus* females were analyzed throughout the study, with an average CW of 10.22 ± 1.48 cm. The monthly GSI is shown in Fig. 1, whose average GSI in *P. pelagicus* is 3.35 ± 2.44 (SD). The highest value of GSI was 8.89% in October 2017 and gradually decreased in November 2017 and December 2017 by 6.08% and 5.32%, respectively. During oogenic production, the data showed that the high value of GSI was 5.53 again in February 2017 (Fig. 1).

3.3 External Morphology and Ovarian Development

In this study, the results revealed that the ovarian structure of *P. pelagicus* was covered by a thin layer of connective tissue, referred to as an “ovarian capsule”. The ovarian parenchyma was found to be composed of several ovarian lobes (or ovarian follicles) (Fig. 2A). Each lobe was divided into two important compartments, including the germinal nest and germinal zone (Fig. 2B). The germinal nest was found closer to the central zone of the lobe (Fig. 2B). Two oocyte stages, the oogonia and pre-vitellogenic stage (Oc1), were noted to be clearly visible inside the germinal nest (Figs. 2C and 2D). The oval shape of Oc1 contained a central nucleus, with one prominent nucleolus, surrounded by basophilic cytoplasm (Fig. 2D). In addition, it was also covered by spindle-shaped follicular cells (Fig. 2D). At the end of this stage, the appearance of the lipid droplet occurred (Fig. 2E). The germinal zones holding different oocytes appeared and moved to the peripheral area of the ovarian lobe (Fig. 2B). The majority differentiation of the secondary-growth oocytes in this zone was classified based on the histological properties and uptake of yolk granules during the early

![Fig. 1](image_url)  Annual variation in gonadosomatic index (GSI) of *Portunus pelagicus* during October 2016 (Oct-16)-September 2017 (Sep-17).
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vitellogenetic stage (Oc2), late vitellogenetic stage (Oc3) and mature oocyte stage (Oc4) (Figs. 2F-2I). The cytoplasm of the Oc2 was observed as a reddish stain due to it beginning to accumulate small yolk granules into the periphery of the oocyte (Fig. 2F). The Oc3 showed that the cell was characterized by a remarkable prominent acidophilic stain containing large yolk granules in the cytoplasm (Fig. 2G). They positively reacted with PAS reaction, indicating the presence of glycoprotein (Fig. 2H). At the same time, some areas of the yolk granules were benignly filled. Finally, the yolk granule of the Oc4 was completely filled, whereas the nuclear feature was not apparent (Fig. 2I). As a result, the assessment of the oocyte of *P. pelagicus* agreed with previous observations [6, 7], for example, *P. trituberculatus* [18] and *Scylla serrata* [19].

Histologically, the ovarian development of *P. pelagicus* can be classified into six stages during the reproductive cycle (stages I, II, III, IV, V and VI), which are closely related to progressively increasing oocytes and cellular organization (Figs. 3 and 4).

3.3.1 Stage I (Immature Stage)

The morphology of the ovary, which is made up of thin tubular organs that appear as a creamy white colour to the naked eye (Fig. 3A), is sometimes difficult to separate from the digestive structure. The structural arrangement of the ovary has a conspicuous H shape. The oogeneic stages, including rounded oogonia and Oc1, had more advanced development in the central zone of the ovarian lobe (Fig. 4A).

3.3.2 Stage II (Early Maturing Stage)

The external morphology of the ovary was visually distinguishable due to it becoming a light yellow colour (Fig. 3B), which was easily differentiable and drastically larger in size when compared with the previous stage. A few oogonia and Oc1, especially in late Oc1 complete with large lipid droplets, were prominently seen (Fig. 4B).

3.3.3 Stage III (Late Maturing Stage)

The ovary became dark yellow in colouration (Fig. 3C), which covered half of the dorsal hepatopancreas (Fig. 1). The ovaries contained very few oogonia, whereas Oc1 was still seen in the central zone of the ovarian lobe. The beginning of Oc2 was greatly developed and had increased in the germinal zone (Fig. 4C).

3.3.4 Stage IV (Early Matured Stage)

The matured ovaries in the crabs were deep yellow to orange in color (Fig. 3D). The initiation of advanced Oc2 development, and especially Oc3, was prominently found (Fig. 4D).

3.3.5 Stage V (Middle Matured Stage)

The ovigerous female crabs were found to be bearing fully matured eggs that were orange in colour (Fig. 3E). Two prominent oogenic stages, Oc3 and Oc4, were observed (Fig. 4E).

3.3.6 Stage VI (Late Matured Stage)

The female crabs were found to be bearing fully matured eggs that were black in colour (Fig. 3F), whereas the histological observations showed that the ovaries were deposited during Oc4 (Fig. 4F).

3.4 Changes in GSI and Ovarian Development during Reproductive Cycle

Accurate measurements of the reproductive cycle allowed the validation of both GSI and ovarian development. By comparing the percentages, it was found that oogenic production in *P. pelagicus* continuously appeared throughout the year (Fig. 5). Ovarian development was regularly found in stage VI from October 2017 until December 2017, with the highest peak in November 2017 (39.96%) in relation to high GSI, while the lowest peak was in December 2017 (10%). Stage V was only observed in November 2017 (13.32%). During October 2017 to February 2018, stage I was found to be present, with the highest peak of GSI recorded in October 2017 (49.5%) and the lowest in December 2017 (10%). Additionally, ovarian development, which includes stages II, III and IV, was found in most months (Fig. 5), implying that oogenic production, especially secondary growth oocytes, took place throughout the year.
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Fig. 2 Light microscopic level showing the ovarian structure and oogenesis of *P. pelagicus*: ovarian lobe (Ol) in the ovary (A); the Ol was composed of the germinal nest (Gn) and germinal zone (Gz) (B); oogenesis was divided into oogonia (Og) (C), pre-vitellogenic (Pv) stage (D, E), early vitellogenic stage (Evs) (F, G, H), late vitellogenic stage (Lvs) and mature oocytes stage (Mos) (I).

Av = atretic oocyte of vitellogenic stage; Fe = follicular cell; Ld = lipid droplet; N = nucleus; nu = nucleolus; Yg = yolk granules.

A-G, I = Harris’s haematoxylin and eosin; H = Periodic’s Schiff reagent.
Evidence from previous observations suggests that the criteria for evaluating oocyte development in *P. pelagicus* have already been widely identified in various publications [3, 5, 20], but the identifications of the different ovarian stages of *P. pelagicus* vary based on geography. For example, four stages of ovarian development (or the ovarian cycle) of *P. pelagicus* were divided based on histological studies and oogenesis [3, 5]. Consequently, the seasonal changes of the ovarian cycle of *P. pelagicus* were categorized into five stages according to varian colour and histological changes of the oocytes [21]. It is possible that the differential criteria and nomenclature for classifying the ovarian development of *P. pelagicus* were based on the developing understanding, experience and qualifications of past researchers studying oocyte feature. The documentation of the ovarian stages of *P. pelagicus* females from Kung Krabaen Bay, Kunsook *et al.* (2014a) [8] first divided the female reproductive cycle into four stages (stages I-IV) over the course of three spawning periods (November-December, February-March and June-August). In comparison, this observation clearly recorded, during October to December, only the peaks or a maximum from two integrated methods during the same period as previous observation [8]. The reasons are unclear as to why these changes only occur during the spawning
period. It is possible that it might be related to the influence of water temperature, as this factor is considered a key role in controlling the ovarian development and spawning of *P. pelagicus* [6, 7, 22, 23]. The high proportion of stage IV and GSI in *P. pelagicus* females during the November to December period might indicate a response to the decrease in temperature (about 29.55 °C), which was different when compared with other periods throughout the year. The hypothesis is that this temperature created a more favourable condition for ovulation and spawning activity. However, further studies should be undertaken to determine this hypothesis under laboratory observation.

![Fig. 4 Ovarian histology of *P. pelagicus* during the reproductive cycle: stage I or ovarian immature (A), stage II or early ovarian maturing (B), stage III or late ovarian maturing (C), stage IV or early ovarian matured (D), stage V or middle ovarian matured (E) and stage VI or late ovarian matured (F).](image)

A-F = Harris’s hematoxylin and eosin.
4. Conclusions

These results provide a clearer picture of the annual oogenesis cycle of *P. pelagicus* inhabiting Kung Krabaen Bay, Thailand, where the cycles occurred throughout the year and accurately within the spawning season (October to December). These results are hence forth important for the hatchery seed production and conservation of *P. pelagicus*. Moreover these results have been used for fishery improvement project (FIP) in blue swimming crab *P. pelagicus* in Thailand particularly measurement in forbidden harvesting the ovigerous female in this period and also should be limit the minimum legal size for catching the female crab in this area.

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