Some Aspects of Reproductive Biology of Blue Swimming Crab (*Portunus pelagicus* (Linnaeus, 1758)) Under Laboratory Conditions

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ABSTRACT

This study was carried out to observe and describe sexual dimorphism, reproductive system, macroscopic and histological gonad development and Gonado Somatic Index (GSI) of blue swimming crab (*P. pelagicus*) during post-spawning, spent spawner and berried female under laboratory conditions. The general sex dimorphism and reproductive system of male and female blue swimming crab were observed similar to be most other decapods crustaceans. The pubertal molt, the abdomen and gonopores of female show changes that are generally accepted as external morphological indications of sexual maturity. Unlike female, the males show prepubertal (loosing of the attachment of the abdominal flap to the cephalothorax) rather than pubertal molt. The ovaries and testes were classified into five and three development stages and the ovarian histology of each stage was characterized. The ovarian stages correlated closely with the Gonado Somatic Index (GSI), the characteristics of ovarian histology and oviposition period.

**Key words:** Blue swimming crab, gonad development, gonado somatic index, portunus pelagicus, reproductive biology

INTRODUCTION

The blue swimmer crab (*P. pelagicus*), has been the object of commercial fishing (Thomson, 1951; Ikhwanuddin and Oakley, 1999; FAO, 2000; Kangas, 2000) and recreational fisheries in Australia and other parts of the world (Kangas, 2000). In the wild for this species has been thoroughly studied; depth distribution patterns (Stephenson, 1962; Kailola et al., 1993; Williams, 1982; Edgar, 1990), population structure (Bryars and Adam, 1999), reproductive cycle (Meagher, 1971; Pillai and Nair, 1973; Potter et al., 1983, 1998), mating behaviour (Smith, 1982; Potter et al., 1983) and development of larvae (Yatsuzuka, 1962; Kurata and Midorikawa, 1975; Bryars, 1977). However, no studies are available on the reproductive biology of blue swimming crab (*P. pelagicus*) under laboratory conditions. The study of crab reproduction can be divided in two different aspects: First, the events related to mating, including the processes which precede and continue the copula
itself. Second, the reproductive cycle of a species, in which information required (e.g., maturity, egg development, the relationship between reproduction and moult cycle) is usually obtained by means of periodic samples of a given population (Gonzales-Gurriaran, 1985).

Research on crustacean reproductive biology is demanded due to the need of maintaining natural fishing stock (Santos, 1994), establishment of a fishery management program (Pinheiro and Fransozo, 1998) and availability of spawners in hatchery.

MATERIALS AND METHODS

Adult male and female, with mean Body Weight (BW) of 53.8-360.8 g and Carapace Width (CW) of 915-158.67 mm, were caught in baited traps from the coastal region of Port Dickson. Immediately after capture, the weight, Carapace Width (CW), distance between the tips of the largest spines) and Carapace Length (CL, midline distance from diastema between the rostral teeth to the posterior carapace edge) of each animal were measured with digital Vernier calipers to nearest 0.05 mL and the male’s gonads were removed. The external sexual characteristic of all animals collected were examined and described.

After being examined for their sexual characteristic, especially non-berried females were disinfected with 100 ppm formalin for 30 min before placing in maturation and hatching tanks, while the male crabs were directly dissected in order to ascertain their gonad development stages.

Non-berried females were randomly stocked in seven units of 1 m³ cylindrical black plastic tanks at maximum density of three crabs per tank. Tanks were provided with substrate of around 15 cm thick sand and adequate aeration. The crabs were maintained in constant water depth of 25-30 cm with salinity of 30-32 ppt, pH 7.77-7.96, temperature 26-27°C and O₂ 7.0-7.3 ppm. Each crab was provided with a shelter made of PVC pipe: 13 cm in diameter and 40 cm in length, to serve as refuge during molting and attack by fellow crabs. Fresh bivalve mollusks were given as food daily at 1700-1800 h and uneaten food was removed every morning.

The observation of oviposition was only made on female crabs and from 21 non-berried females kept in maturation tanks for a month. After completing the process (oviposition, incubation and hatching period), the spent spawners were placed again into each of the maturation tanks containing the 3 females. For observation of ovarian development, the crabs were collected randomly and checked regularly once in every ten days within a 40 day period.

Gonad development was determined using two criteria: Gonadosomatic Index (GSI) (Atz, 1975; Pudadera and Primavera, 1981) and gonad morphologic characteristic (Kumar et al., 2000; De Lestang et al., 2003). To calculate GSI, whole preserved gonads are rinsed in freshwater, blotted dry, weighed to the nearest gram. Gonad wet weight is then divided by the wet weight of crab calculated by the equation:

\[
GSI = \frac{WWG}{WWC} \times 100
\]

Where:
- GSI = Gonadosomatic index
- WWG = Wet weight of gonad (g)
- WWC = Wet weight of crab (g)
Developing spermatozoa and oocyte were observed histologically for the gonads undergoing the maturation process. The gonads were weighed and their general appearance was recorded. Each gonad was divided into four regions for male and three regions for female. The separate regions were fixed in Bouin’s solution, sectioned at 6 µm thick and stained with hematoxylin and eosin for histological analysis. The predominant stage of development for the different regions was designated as the maturation stage of the whole ovary.

The data of sexual dimorphism, reproductive system, macroscopic and histological gonad development and Gonadosomatic Index (GSI) are analyzed descriptively.

RESULTS

Sexual dimorphism: Morphological observation on male and female blue swimmer crab (*P. pelagicus*), showed that they could be differentiated through their secondary sexual characteristics. This species is dioecious and male and female are easily identified using the following morphological features:

- The females is mottled brown (Fig. 1a), while males display blue colouration (Fig. 1b)
- The females with triangular (Fig. 1c) and suboval (Fig. 1d) abdomens were considered juvenile and adult crabs, respectively. In males, both juveniles and adults specimens have inverted “T” shaped abdomens (Fig. 1e)
- The females have four pairs of pleopods (or swimmerets) on abdominal segments 2 through 5 (Fig. 1f). The first article, or coxa, of a pleopod is attached to the body by a soft and flexible articulating membrane. The coxa is small and poorly calcified but the next article, the basis, is large and conspicuous. Two rami, the exopode and endopod, arise from the basis. Male have only two pairs of pleopods and are located anteriorly on the abdomen, on segments 1 and 2 (Fig. 2a). Both functions in the transfer of sperm to the female during copulation. The long, curved, tubular first pleopod is the gonopod. It is not the penis, instead an intermittent organ used to deliver spermatophores to the female gonopore. The second pleopod is much shorter and functions as a piston to push spermatophores through the hollow core of the gonopod
- The gonopores of the females are situated on the coxae of the third pair of walking legs (Fig. 2b)

Reproductive system

Female reproductive system: The female reproductive system consists of a pair of ovary. The ovary is H-shaped and located dorsal to the hepatopancreas and extends posteriorly along each side of the hind-gut (Fig. 3a, b). The most anterior part of the ovary is found at the base of the large ninth antero-lateral spine. The ovary curve along the antero-lateral margin and is bound in place by a connective tissue envelope which contain many black chromatophores which are particularly evident in the dissection of live specimen. As the ovaries curve medially from the lateral spines, they run lateral to the stomach, pass medial to the tendon of the adductor muscles of mandible and are then directed posteriorly. The commissure passes ventral to the posterior adductor muscles of the stomach. Posterior to the commissure, the ovary passes ventral to the pericardium and extends posteriorly along the medial edges of the endophragmatic skeleton. The entire ovary is bound by fibrous connective tissue which serves to separate the organ from the surrounding hemocoel. In general, the right ovary extended farther posteriorly than the left. There may be small lateral protrusions of the ovary near its end at the first abdominal segment.
Male reproductive system

Testes: The testes, like the ovaries, are paired organs medially interconnected by a commissure so that they approximate the shape of a letter “H” (Fig. 3). Their position is just dorsal to digestive gland and under the hypodermis of the carapace (Fig. 3c). Extending from the middle of the postero-lateral border of the carapace, the testis follows the curve of the antero-lateral border of the carapace and passes lateral to the stomach and ends medial to the tightly coiled anterior vas deferens. The commissure arises on the medial border of the testis and runs along the testis for several millimeters before passing posterior to the stomach and ventral to the posterior gastric muscle.
Fig. 2(a-b): Sexual dimorphism of blue swimming crab *P. pelagicus*: (a) Female-pleopod or swimmeret (Fm-pl or sw) and (b) Female-gonopore (Fm-gp), female-black margin (Fm-b lcm)

Fig. 3(a-d): Reproductive system of blue swimming crab (*Portunus pelagicus*), (a) Paired ovaries (OV) located in the dorsal to the hepatopancreas and extends posteriorly along each side of the hind-gut, (b) Female reproductive system: Ovary (OV), Ovary commissure (OC), (c) Paired testes (T) located in the dorsal to the hepatopancreas and extends posteriorly along each side of the hind-gut and (d) Male reproductive system, T: Testes, TC: Testicular commisure, A VD: Anterior vas deferens, M VD: Median vas deferens, P V D: Posterior vas deferend
Vas deferens: The vas deferens extends from the posterior end of the testis through the thoracic cavity and the pereiopodal musculature of the 8th thoracic segment where it ends in the penile papilla on the coxa of the 5th pereiopodal. The vas deferens is divisible into three major portions which differ in form and function (Fig. 3d). These are termed here anterior, median and posterior vas deferens to conform to nomenclature of Cronin (1974) and abbreviated as AVD, MVD and PVD.

Macroscopic and histological gonad development

Female: On the basis of macroscopic and histological studies, the stages in maturation of oocytes may be characterized briefly as follows:

C Immature (stage I): The immature ovary is white/translucent, small and flattened and not extending into hepatopancreas. Individual oocytes are not visible. Anterior region is small and does not displace the hepatopancreas. The posterior section, located in the cardiac and intestinal regions, forms two parallel lobes. Histologically, the immature ovary stage consists of oogonia, differentiating oogonia and previtellogenic oocyte (Fig. 4).

C Early maturing (stage II): Ovaries are light yellow/orange, larger than stage I ovaries and slightly nodulated. Individual oocytes are not visible. Ovaries and hepatopancreas size ratio approximately 1:2 and the two lobes of the posterior region are starting to become convulated. Histologically, the ovary in stage II consists mainly of previtellogenic and vitellogenic oocytes (Fig. 5). The second growth phase is also characterized by the formation and accumulation of yolk. The first indication of yolk formation is the presence of vesicles in the periphery of the cytoplasm of oocytes.

C Advanced maturing (stage III): Ovaries are yellow/orange, large and nodulated. Individual oocytes are just visible through ovary wall. Anterior region displaces the hepatopancreas and the central and posterior regions occupy almost all of the space in the gastric, posterior and the intestinal cavities. Vitellogenic oocytes were mainly observed in the ovary in stage III (Fig. 6). From histological examination, in the early part of this stage the yolk vesicle occupies the entire

Fig. 4: Ovarian section at stage I stained with hematoxylin and eosin in immature female of blue swimming crab (*Portunus pelagicus*). OG: Oogonia, PO: Previtellogenic oocyte
Fig. 5: Ovarian section at stage II stained with hematoxylin and eosin in post spawning female of blue swimming crab (*Portunus pelagicus*). PO: Previtellogenic oocyte, EVO: Early vitellogenic oocytes, YV: Yolk vesicle

Fig. 6: Vitellogenic oocyte in ovarian section at stage III stained with hematoxylin and eosin in post spawning of blue swimming crab (*Portunus pelagicus*). FC: Follicle cells, N: Nucleus, Nu: Nucleolus

Ooplasm and the appearance of each oocyte is characterized by a granular appearance. The granular first developed close to the follicular layer. The follicular layers are fairly well developed.

**Mature (stage IV):** Ovaries are dark yellow/orange, highly nodulated, larger than the hepatopancreas and occupying all cephalothoracic cavities. Individual oocytes are clearly visible through the ovary. Mature oocytes occur immediately before ovulation. Histologically, the ovary is dominated by mature ova which appear granulated due to the high concentration of yolk globules (Fig. 7). Vitellogenesis is essentially complete at this stage and the chorionic membrane surrounding each ovum is conspicuous. The oocytes are characterized by the migration of the nucleus to the animal pole and fusion of the yolk globules and oil droplets. The germ strand is
Fig. 7: Late vitellogenic or maturing oocyte and surrounding follicle cells in ovarian section at stage IV stained with hematoxylin and eosin in berried female of blue swimming crab (*Portunus pelagicus*). N: Nucleus, MO: Maturing oocyte, FC: Follicle cells

usually obscured by the tightly packed mature ova and accessory cell are present only proximal of the oocytes still undergoing vitellogenesis. The membrane enclosing the egg proper is comparatively thick.

C **Spawned or redeveloping ovary (stage V):** Ovaries are light yellow, tans or yellow-orange but not bunched up and are flaccid. A few residual orange eggs can sometimes be seen through the ovary wall. Histologically, the germ strand is well defined and oogonia and developing oocytes radiating outwards from this region. Visible degeneration of the oocytes occurs, mostly to mature unspawned ova. The yolk globule contracts irregularly, beginning from the edge of follicular envelope and moving toward the center. The follicular envelope begins to disintegrate and its outer surface becomes irregular. It eventually ruptures, invasion of the interior of the oocyte begins and the yolk is phagocytosed by granulose cells which undergo hypertrophy (Fig. 8).

**Male:** The gonad development of the wild male crab was examined both macroscopically and histologically. The morphological examination of the wild male crabs revealed that male’s gonad had only up to stage III (mature I). The staged-mature-I of male’s gonad is opaque, white and swollen. Anterior Vas Deferens (AVD) and Middle Vas Deferens (MVD) distended and white and Posterior Vas Deferens (PVD) distended and convoluted but still opaque (Fig. 3c and d). Histologically, the proliferation of spermatogenic cell continued and the abundance of spermatozoa in the lumen were observed (Fig. 9-11).

**Gonadosomatic index (GSI):** The carapace width and the carapace length of the blue swimming crabs ranged from 94.15-158.67 and 42.88-74.03 mm, respectively in males and 106.31-143.15 and 48.69-69.35 mm in females. The body weight varied from 53.80-360.80 g and 95.30-228.40 g, respectively in the males and females. The gonad weight ranged from 2.18-13.84 g in males and
DISCUSSION

Sex dimorphism: The general sex dimorphism of male and female P. pelagicus was similar to that described for blue crab (Callinectes sapidus) (Hill et al., 1989), dungeness crab (Cancer magister) (Pauley et al., 1986), speckled swimming crab (Arenaeus cribrarius) (Pinheiro and Fransozo, 1998)
Fig. 10: Cross-section of anterior vas deferens (AVD) from matured male blue swimming crab (*Portunus pelagicus*) stained with hematoxylin and eosin showing lobules filled with spermatozoa mass (SPM) at stage III (matured-I). AVD: Anterior vas deferens, SPM: Spermatozoa mass.

Fig. 11: Cross-section of median vas deferens from matured male blue swimming crab (*Portunus pelagicus*) stained with hematoxylin and eosin filled with spermatozoa mass (SPM) at stage III (matured-I). SPM: Spermatozoa mass.

and other Portunidae. Specifically, in females, the characteristics of this species were closely associated with regards the development of secondary characteristic and physiological maturity. According to Hartnoll (1969) the abdomen and gonopores show changes following pubertal molt that are generally accepted of sexual maturity. Change in the gonopores following pubertal molt and accompanying mating have been reported for the three species such as *Geryon fenneri* (Hartnoll, 1968) *G. quinquidens* (Hafner, 1977) and *G. maritae* (Melville-Smith, 1987). In addition, *G. fenneri* exhibited the simple pattern of gonopores described by Hartnoll (1968), with three distinct types recognized. Type A gonopores which are narrow and slitlike are present on...
Table 1: Gonadosomatic indexes of 18 male blue swimming crab (*P. pelagicus*), with their respective body weight, carapace width, carapace length, gonad weight and maturity stage

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<th>CL (mm)</th>
<th>GW (g)</th>
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SE: Standard errors, Maturity stage 3 = III, BW: Body weight, CW: Carapace width, CL: Carapace length, GW: Gonad weight, MS: Maturity stages and GSI: Gonadosomatic index

sexually immature animals. Type B gonopores which followed the pubertal molt are elongated and ovoid in shape, while type C which is a modification of the type B differed only in that the gonopore is more elongated and gaping as a result of the mating during the immediate post molt period. In addition, type C gonopores commonly exhibit a blackened margin due to abrasion by the male pleopods during mating. Similarly, this pattern was also observed in blue swimming crab (*P. pelagicus*).

Reproductive system: The general reproductive system of the female and male gonad in *P. pelagicus* were similar to other decapod crustacean. Details of the anatomy of the gonad have been presented for *Portunus sanguinolentus* (Ryan, 1965a, b), *Geryon quinquepennis* (Hafner, 1977), *Scylla serrata* (Uma and Subramoniam, 1984), *G. maritae* (Melville-Smith, 1987), *G. fenneri* (Erdman and Blake, 1988) and *P. pelagicus* (De Lestang et al., 2003).

Macroscopic and histological gonad development: The stages of ovarian development identified in this study closely resembled those described in other studies for *P. pelagicus* (Kumar et al., 2000; De Lestang et al., 2003), *P. sanguinolentus* (Ryan, 1965a, b). In general, ovarian development could be separated into distinct stages: (1) Initial oocyte proliferation and growth (corresponding to ovarian stages I and II), followed by (2) Vitellogenesis. Vitellogenesis could also be divided into two stages, (a) Ovarian stage 3 and (b) Ovarian stages 4 which culminated with oviposition (Stewart et al., 1997).
Table 2: Gonadosomatic indexes of 18 female blue swimming crabs (*Portunus pelagicus*), with their respective body weights, carapace width, carapace length, gonad weight and maturity stage

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</table>

SE: Standard errors, maturity stage (MS) 2: II, pale yellow, 3: III, yellow, 4: IV, deep yellow to orange (Examination for ovarian development was done macroscopically through the dorsal side of the crab), a: Non berried female-from the wild, b: Non berried-reared under laboratory, c: Post spawning, d: Spent spawn, BW: Body weight, CW: Carapace width, CL: Carapace length, GW: Gonad weight, MS: Maturity stages and GSI: Gonadosomatic index

The male gonad development pattern, displayed direct differentiation into male, which matched the exact description of differentiated gonochoristic (De Lestang et al., 2003), similar to that of the *P. sanguinolentus* (Ryan, 1965a, b), *S. serrata* (Uma and Subramoniam, 1984) and *Emerita holthuisi* (Nagabushanam and Kulkarni, 1997).

Based upon observation of both macroscopic and histological studies, all of male crabs caught from wild were predominantly with stage III ovaries; none developed to stage I and II. This indicated that all non berried females used in the present study may have previously undergone mating and contained spermathecae. Besides, the presence of male in the third stage also indicated that this period was known as the spawning season. By this reason the following study on the multiple spawning and oviposition investigation could be well observed in the female crabs caught this period.

**Gonadosomatic index (GSI):** The gonadosomatic index (GSI) of the gonads has been used as an indication of spawning in vertebrates (Berry, 1964; Ahsan, 1966). The GSI of the gonads of *P. pelagicus* was calculated and expressed as a percentage of the weight of gonad to that of the body weight. On the whole mean GSI crab female from stage II to IV revolved at around 3.85-11.99, whereas GSI crab male varied from 2.53-6.84 at stage III. At the same maturation rate (III) or GSI value, female crab was bigger than male crab, probably caused by the increase in the ovaries weight larger than the growth of testes. Nikolsky (1963) said that the ovaries weight was around 15% from the body weight but Scott (1979) reported the values between 15 and 30%.
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