



Size distribution and monthly variation of ovarian development for the female blue swimmer crab, *Portunus pelagicus* in Beibu Gulf, off south China

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Summary: The present study was conducted to investigate the size distribution and seasonality variation of ovarian development for females of the blue swimmer crab, *Portunus pelagicus*, from Beibu Gulf, China. The results show that, based on pubertal moult, gonadosomatic index (GSI), morphology and histological configurations, the ovarian development cycle of *P. pelagicus* can be divided into five stages; Stage I, dominated by oogonia with some previtellogenic oocytes and endogenous vitellogenic oocytes; Stage II, dominated by endogenous vitellogenic oocytes with some previtellogenic oocytes and oogonia; Stage III, dominated by exogenous vitellogenic oocytes; Stage IV, of nearly mature oocytes; and Stage V, of mature oocytes with fully ripened ovary. GSI increased significantly during the ovarian development ($P < 0.05$), while the hepatosomatic index (HSI) appeared to decrease. Thus, a significant negative correlation was found between GSI and HSI ($P < 0.01$). The smallest carapace width of a mature female and that at which 50% of females reach maturity were 79.2 mm and 108.2 mm, respectively. The carapace width and body weight of the dominant size class ranged between 86 and 115 mm and 40 and 120 g, respectively. Furthermore, the highest GSI of female *P. pelagicus* was found in March, while the peak of ovigerous females was found in April.

Keywords: blue swimmer crab; ovarian development; size at maturity; seasonality.

Distribución del tamaño y variación mensual del desarrollo ovárico del cangrejo azul hembra, *Portunus pelagicus* en el golfo de Beibu, frente al sur de China

Resumen: Este estudio se realizó para investigar la distribución del tamaño y la estacionalidad de desarrollo ovárico del cangrejo azul hembra, *Portunus pelagicus* en el golfo de Beibu, China. Los resultados muestran que, sobre la base de la muda de la pubertad, el GSI (índice gonadosomático), la morfología y las configuraciones histológicas y el ciclo del desarrollo ovárico de *P. pelagicus* puede dividirse en cinco etapas. Etapa I: La fase de oogonia (OG) dominaba con algunos ovocitos previtelogénicos (PR) y ovocitos vitelogénicos endógenos (EN); Etapa II: fase de EN dominaba con algunos PR y OG; Etapa III: la fase de ovocitos vitelogénicos exógenos (EX) era dominante; Etapa IV: fase de ovocitos casi maduros (NO); Etapa V: fase de ovocitos maduros (MO) y maduración completa del ovario. El índice gonadosomático (GSI) aumentó significativamente durante el desarrollo del ovario ($P < 0.05$), mientras que el índice hepatosomático (HSI) parecía disminuir. Por lo tanto, se encontró una correlación negativa significativa entre GSI y HSI ($P < 0.01$). La menor anchura del caparazón (CW) de una hembra madura y CW del 50% de las hembras maduras eran 79.2 mm y 108.2 mm, respectivamente. La CW y el peso corporal de la clase de tamaño dominante oscilaban entre 86-115 mm y 40-120 g respectivamente. Por otra parte, el GSI de la hembra más grande de *P. pelagicus* se encontró en marzo, mientras que el pico de las hembras ovígeras se produjo en abril en la población del golfo de Beibu.

Palabras clave: cangrejo azul; desarrollo ovárico; talla de madurez; estacionalidad.

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INTRODUCTION

The blue swimmer crab, *Portunus pelagicus* Linnaeus 1758, is widely distributed in the Indo-Pacific, particularly in tropical and subtropical waters, and supports important fisheries in many countries within the region (Xiao and Kumar 2004, Romano and Zeng 2008). Due to high demand, unique taste and availability in all seasons, the species is popular and has a high market demand (Sumpton et al. 1994, Wu et al. 2010). In China, it is mainly distributed in the East Sea and South Sea of western Pacific (Wang et al. 2001) and the annual fishing production is around 80000 t of *P. pelagicus* each year (Bureau of Fisheries 2010). Beibu Gulf is the historical centre for the blue swimmer crab fishery in China; with annual landing production estimated around 20000 t per year (Wang et al. 2001, Bureau of Fisheries 2010). However, wild *P. pelagicus* has been overfished, and in the future, the expansion of *P. pelagicus* output depends on aquaculture and stock enhancement programmes (Maheswarudu et al. 2008, Dixon and Hooper 2009). Therefore, a better understanding of its reproductive biology would directly benefit the fisheries management as well as artificial propagation of *P. pelagicus* (Stewart et al. 2007, Johnson et al. 2010).

Many studies have been conducted on the reproductive biology of female *P. pelagicus*, including size of sexual maturity (Shields and Wood 1993; Smith et al. 2004; Johnson et al. 2010), changes of external features of the ovary (gross morphology and colouration) (Sumpton et al. 1994), anatomical changes of spermatheca (Bawab and El-sherief 1988), histological changes of ovary (Stewart et al. 2007), seasonal fluctuations of gonadosomatic index (GSI) (Pillay and Nair 1973, Sumpton et al. 1994), temporal changes in the proportion of gravid females (Shields and Wood 1993, Sukumaran and Neelakantan 1998, Kumar et al. 2003), fecundity and egg batches (Johnson et al. 2010, De Lestang et al. 2003a). However, the criteria of ovarian staging of *P. pelagicus* is inconsistent and unclear among the different publications (Sumpton et al. 1994, Stewart et al. 2007), making it difficult to apply these ovarian staging criteria for fisheries management and to compare the results from different research reports (Stewart et al. 2007). The correlation of sexual maturity with gonadosomatic index (GSI), external features and ovarian histology could help wild fisheries managers and aquaculture specialists make informed decisions about their catches and extend the knowledge to breeding programmes of *P. pelagicus*. Therefore, a comprehensive method for ovarian staging, such as a combination of GSI, external features and ovarian histology, is required for the female *P. pelagicus*.

Previous studies on the reproductive biology of female *P. pelagicus*, have reported regional and area-specific differences in size at sexual maturity, timing and length of female ovarian development, and spawning season (Shields and Wood 1993, Sumpton et al. 1994, Smith et al. 2004, Xiao and Kumar 2004, Johnson et al. 2010). Although the Beibu Gulf population of *P. pelagicus* supports the largest *P. pelagicus* fishery

(20% of total landings) in China (Wang et al. 2001), no available publications could be found on female reproductive biology for the Beibu Gulf population of *P. pelagicus*.

The present study of the *P. pelagicus* population in Beibu Gulf was conducted with the aim of determining the following: (1) changes in gonadosomatic index (GSI), hepatosomatic index (HSI), gross morphology and histological features of the ovary during ovarian development; (2) the correlations of GSI and HSI, oocyte diameter and GSI; (3) size at ovarian maturity and pubertal moulting; (4) size distribution and frequency for the females after pubertal moulting; (5) seasonality of ovarian development and the percentage of ovigerous females.

MATERIALS AND METHODS

Crab sampling

Monthly samples of *P. pelagicus* were collected from the Beibu Gulf, near the northwest coast of Hainan Island (109°45'E, 19°58'N, Fig. 1) from September 2007 to May 2008. No samples were obtained from June to August 2008 due to the closed fishing season (May 16 to August 15). Thirty to seventy random female crabs were taken each month. All crabs were measured for carapace width (CW, including lateral spines) and body weight (BW). The live females of above 75 mm CW (<75 mm is juvenile, Wang et al. 2001) were collected every month and air-lifted to the laboratory at Shanghai Ocean University, Shanghai, China.

Crab dissection and ovarian staging

In the laboratory, ovaries and hepatopancreas were dissected from the crabs. Their total weight was recorded and their GSI and HSI were calculated as the percentage of tissue wet weight relative to body wet weight. Photographs of the dissected ovary were taken using a digital camera (Canon Powershot A550). A small portion of ovaries were removed from each dissected ovary and fixed in Bouin's solution for 24

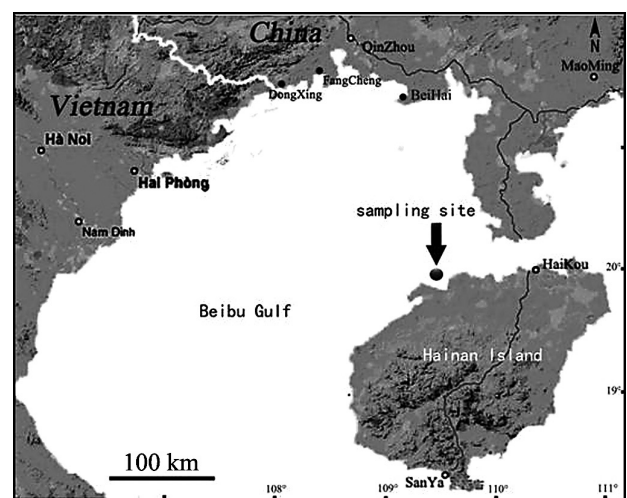


Fig. 1. – Map of Beibu Gulf, showing the sampling site.

hours. The ovarian tissues were then progressively dehydrated in ascending concentrations of ethanol solutions and the dehydrated specimens were cleared with xylene before being embedded in paraffin wax (melting point: 56-58°C). Glass slides of ovaries were prepared (5-7 µm) (Leica RM 2016, Leica Microsystems Inc., Bannockburn, IL, USA). The sections were then stained with haematoxylin-eosin, and histological observations and measurements were made under a light microscope (Olympus BX-41, Olympus Corporation, Shinjuku-ku, Tokyo, Japan). Microphotographs were taken with a digital camera (Nikon IDX1200, Nikon Co., Shinagawa-ku, Tokyo, Japan) equipped with an image manager software (Image-Pro Plus 6.0, Media Cybernetics Inc, Bethesda, MD, USA). Based on Wu et al. (2007): Gametocytes were classified into six categories: oogonia (OG), previtellogenic oocyte (PR), endogenous vitellogenic oocyte (EN), exogenous vitellogenic oocyte (EX), near-mature oocyte (NO) and mature oocyte (MO). The diameter, nucleus and nucleolus of each type of gametocyte was then measured under a microscope (Olympus BX-41) while the nucleo-cytoplasmic ratio (NR) was calculated using the formula $NR = \text{Volume of nucleus} / \text{Volume of cell}$, where volume = $0.523 \times W^2 \times L$ (W, maximum width; L, maximum length) (Wu et al. 2007). Thirty to fifty oocytes from each female were measured for various parameters and the relative frequency of the most advanced oocytes appearing in the ovarian sections became an important criteria for ovarian staging (Quinitio et al. 2007). The calculation for the percentage of each gametocyte types at each ovarian stage was based on 8-13 replicate females and ten slides were observed and calculated under the microscope for each female.

Estimation of size at ovarian maturity

A total of 324 females were used to examine size at ovarian maturity. The females with vitellogenic ovaries were considered to be mature individuals (ovarian developmental stages III-V; detailed staging information is shown in Table 2). To estimate the size at first maturity, the minimum CW was recorded for the females with the oval-shaped pleonal flap, which indicated that the females had finished the pubertal moult (Johnson et al. 2010). The CW at which 50% (CW_{50}) of females were sexually mature was estimated by fitting a logistic regression curve to the percentage of mature *P. pelagicus* for each size class of 10 mm CW, as described by Johnson et al. (2010).

Statistical analysis

Data are presented as mean \pm standard error (SE). The homogeneity of variance of data was tested with Levene's test. When necessary, arcsine-square root or logarithmic transformation was performed prior to analysis. Statistical analysis was conducted using one-way analysis of variance (ANOVA) and Tukey's multiple range test was used to detect significant differences ($P < 0.05$). When a normal distribution and/or homogeneity of variances was not achieved, data were subjected to the Kruskal-Wallis H non-parametric test, followed by the Games-Howell non-parametric multiple comparison test. All analyses were performed using an SPSS statistic package (version 12.0).

RESULTS

Ovary-morphological and histological changes during maturation

Six types of gametocytes of different developmental stages, i.e. OG, PR, EN, EX, NO and MO, were observed during the ovary maturation of *P. pelagicus* (Fig. 2). The details of cell diameter, nucleus diameter, nucleocytoplasmic ratio and volume of the six different-type gametocytes for female *P. pelagicus* are presented in the Table 1. With the development of germinal cells, the cell diameter, nucleus diameter and oocyte volume all increased substantially, but a significant decrease in the short diameter of the nucleus was found when oocytes developed from NO to MO. Meanwhile, the nucleocytoplasmic ratio also showed a significant decrease from 0.56 to 0.0004 with the development from OG to MO ($P < 0.01$). Based on the pubertal moult, gonadosomatic index (GSI), gross external morphology and histological changes in the ovaries, the ovarian maturation of *P. pelagicus* can be divided into five stages (Table 2).

Stage I: Most females (>90%) in this stage were yet to undergo pubertal moult, with a triangular-shaped pleonal flap (Fig. 3A). The ovary was small and slightly convoluted, appearing transparent or translucent (Fig. 3C). In this stage, the ovary was sometimes difficult to recognize and was separated from the hepatopancreas. The GSI of stage I ovaries ranged from 0.02 to 0.30. Histological examination revealed that the dominant types of gametocytes in stage I ovaries OG (61.00%), PR (25.93%) and EN (11.96%). Many follicle cells were found to be distributed in the ovarian lobes and the interspace between ovarian lobes (Fig. 4A). A number of PR

Table 1. – Various parameters of different types of gametocyte cells observed during the ovarian maturation of *Portunus pelagicus*. Values are presented as mean \pm SE. Different superscript letters within the same row indicates significant differences ($P < 0.05$).

Parameter	Oogonia	Previtellogenic oocyte	Endogenous vitellogenic oocyte	Exogenous vitellogenic oocyte	Nearly mature oocyte	Mature oocyte
Long diameter of oocyte (µm)	11.62 \pm 0.14 ^a	22.62 \pm 0.46 ^a	58.58 \pm 2.01 ^b	115.65 \pm 2.79 ^c	237.67 \pm 8.12 ^d	310.84 \pm 5.04 ^e
Short diameter of oocyte (µm)	10.37 \pm 0.18 ^a	17.51 \pm 0.19 ^a	41.29 \pm 1.62 ^b	86.12 \pm 2.08 ^c	173.82 \pm 5.40 ^d	237.48 \pm 4.32 ^e
Long diameter of nucleus (µm)	9.61 \pm 0.19 ^a	15.28 \pm 0.45 ^b	25.27 \pm 0.94 ^c	32.34 \pm 1.47 ^d	28.81 \pm 1.13 ^{cd}	26.31 \pm 1.20 ^c
Short diameter of nucleus (µm)	8.52 \pm 0.16 ^a	12.74 \pm 0.26 ^b	20.61 \pm 0.88 ^c	7.60 \pm 0.35 ^d	19.93 \pm 0.84 ^c	15.92 \pm 0.69 ^b
Nucleocytoplasmic ratio	0.56 \pm 0.073 ^f	0.36 \pm 0.071 ^e	0.12 \pm 0.059 ^d	0.03 \pm 0.01 ^c	0.002 \pm 0.001 ^b	0.0004 \pm 0.0003 ^a
Oocyte volume ($\times 10^5$ µm ³)	0.007 \pm 0.000 ^f	0.04 \pm 0.01 ^e	0.59 \pm 0.07 ^d	4.68 \pm 0.29 ^c	41.75 \pm 3.56 ^b	93.88 \pm 4.39 ^a

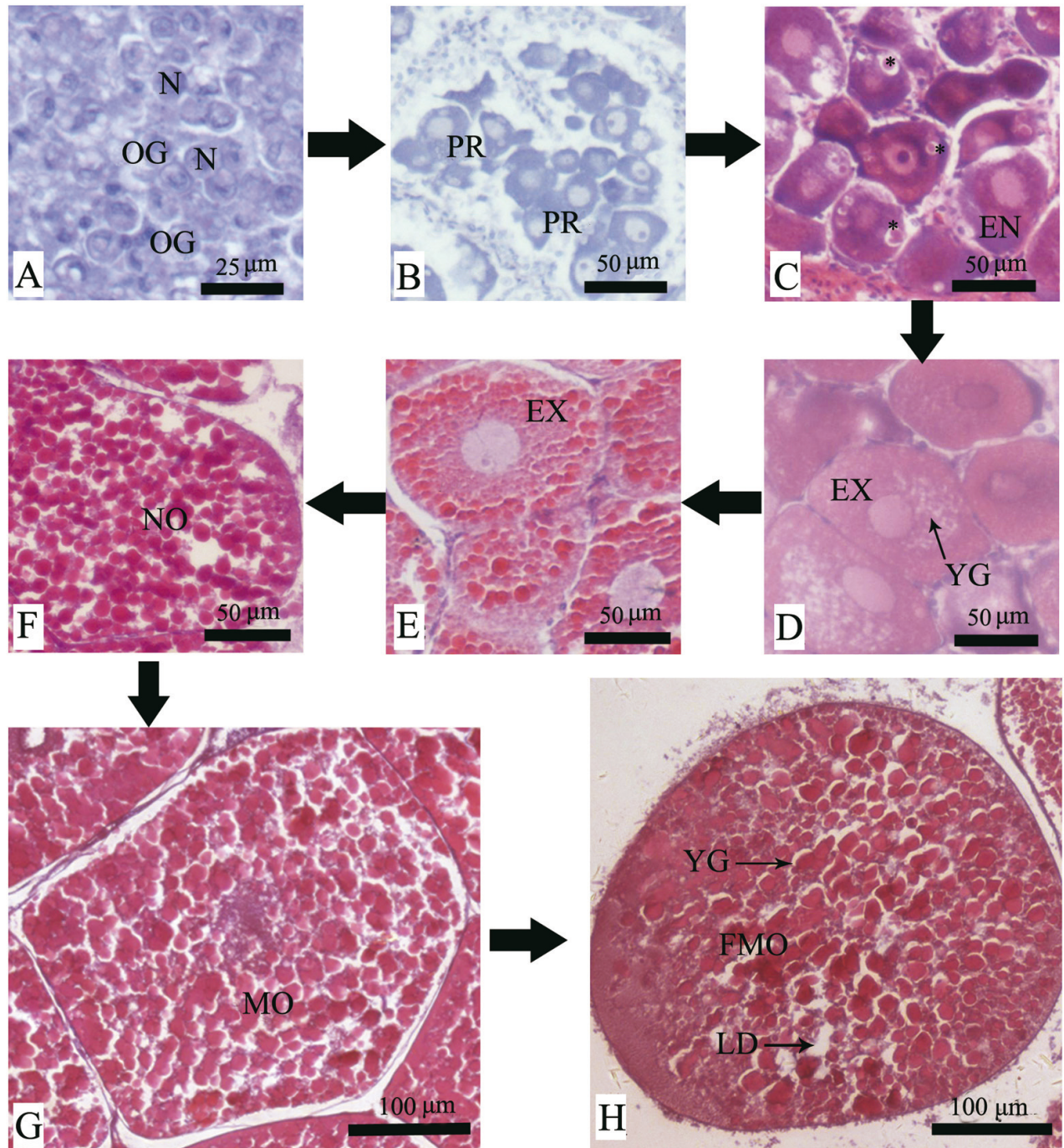


Fig. 2. – Histology of six types of oogenetic cells during the ovarian development of *Portunus pelagicus*. A, oogonia (OG), with basophilic chromosomes condensed in the nucleus (N); B, previtellogenic oocyte (PR), having bluish cytoplasm and without yolk granules; C, endogenous vitellogenic oocyte (EN), with some eosinophilic components (*); D, early exogenous vitellogenic oocyte (EX), showing eosinophilic cytoplasm with many small yolk granules (YG); E, later EX, showing the large yolk granules (YG) and lipid droplet (LD) in cytoplasm; F, nearly mature oocyte (NO); G, mature oocyte (MO), with many large yolk globules (YG); H, free mature oocyte (FMO), which was separated from the follicle cells.

were surrounded by follicle cells, which were observed at the end of stage I (Fig. 4B). The cytoplasm was basophilic for all gametocytes types in this stage.

Stage II: At this ovarian stage most females had undergone pubertal moult and had an oval pleonal flap (Fig. 3B). The ovary colour had changed from milky-white to off-white (Fig. 3D) and two parallel ovary mid-lobes were visible to the naked eye. The GSI of stage II ovaries ranged from 0.27 to 0.54. The dominant

oocyte types of stage II ovaries were EN (40.26%), PR (31.34%) and OG (21.12%) (Fig. 4C). In the same follicle/germinal zone (a small spherical group of cells), the germ cells (EN) in the periphery of the zone had a more advanced development than the gametocytes in the middle zone. In this stage, the cytoplasm had become slightly eosinophilic due to the presence of small yolk globules, and the follicle cells had started to migrate to the periphery of germ zone.

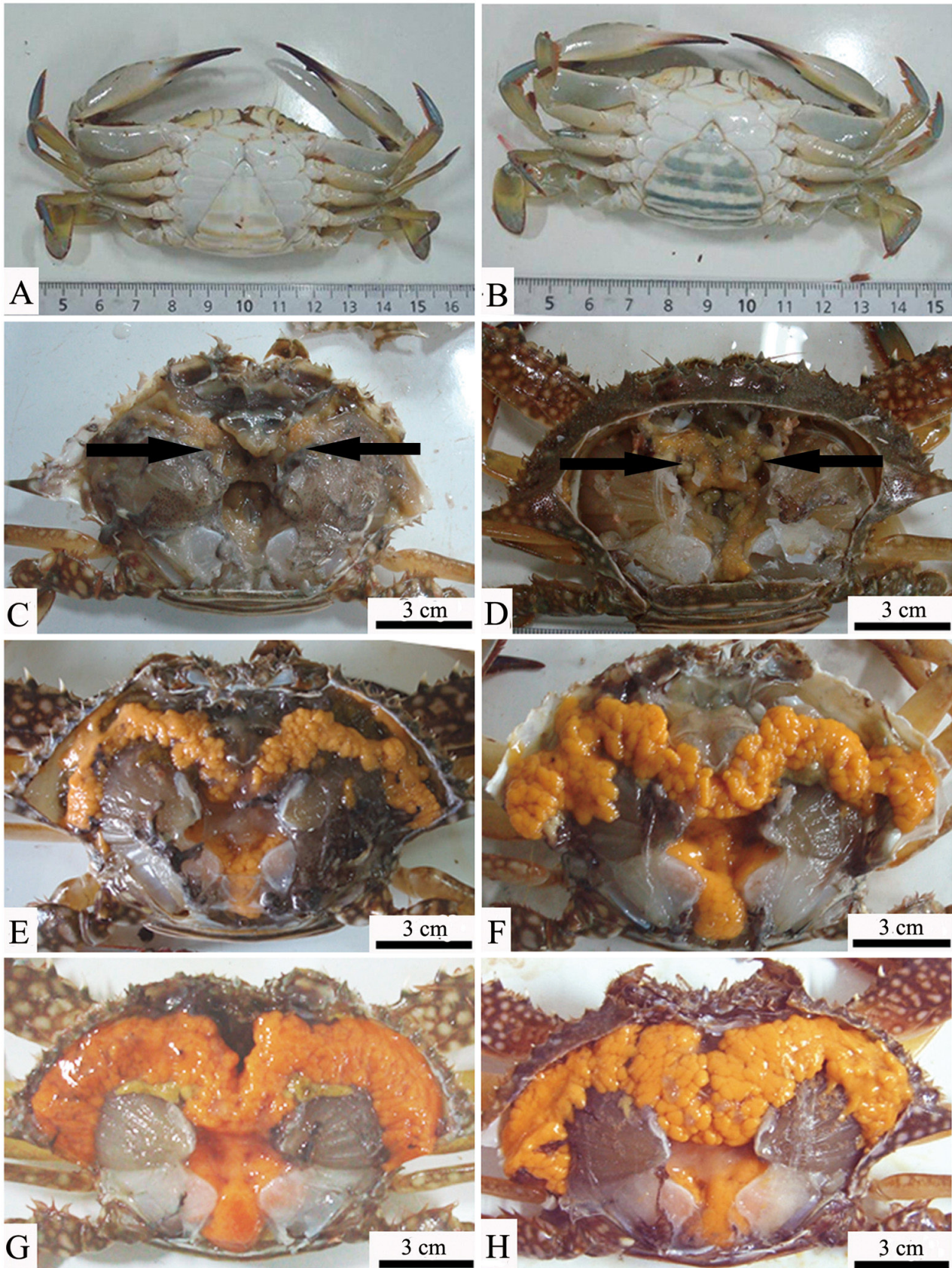


Fig. 3. – Changes in the abdominal shape and different stages of ovarian development in *Portunus pelagicus*. A, the abdominal shape of female *Portunus pelagicus* before puberty moult, showing a triangular abdomen; B, the abdominal shape of female *Portunus pelagicus* after puberty moult, showing a U-shaped abdomen; C, stage I ovary (arrow); D, stage II ovary (arrow); E, early stage III ovary; F, late stage III ovary; G, stage IV ovary; H, stage V ovary.

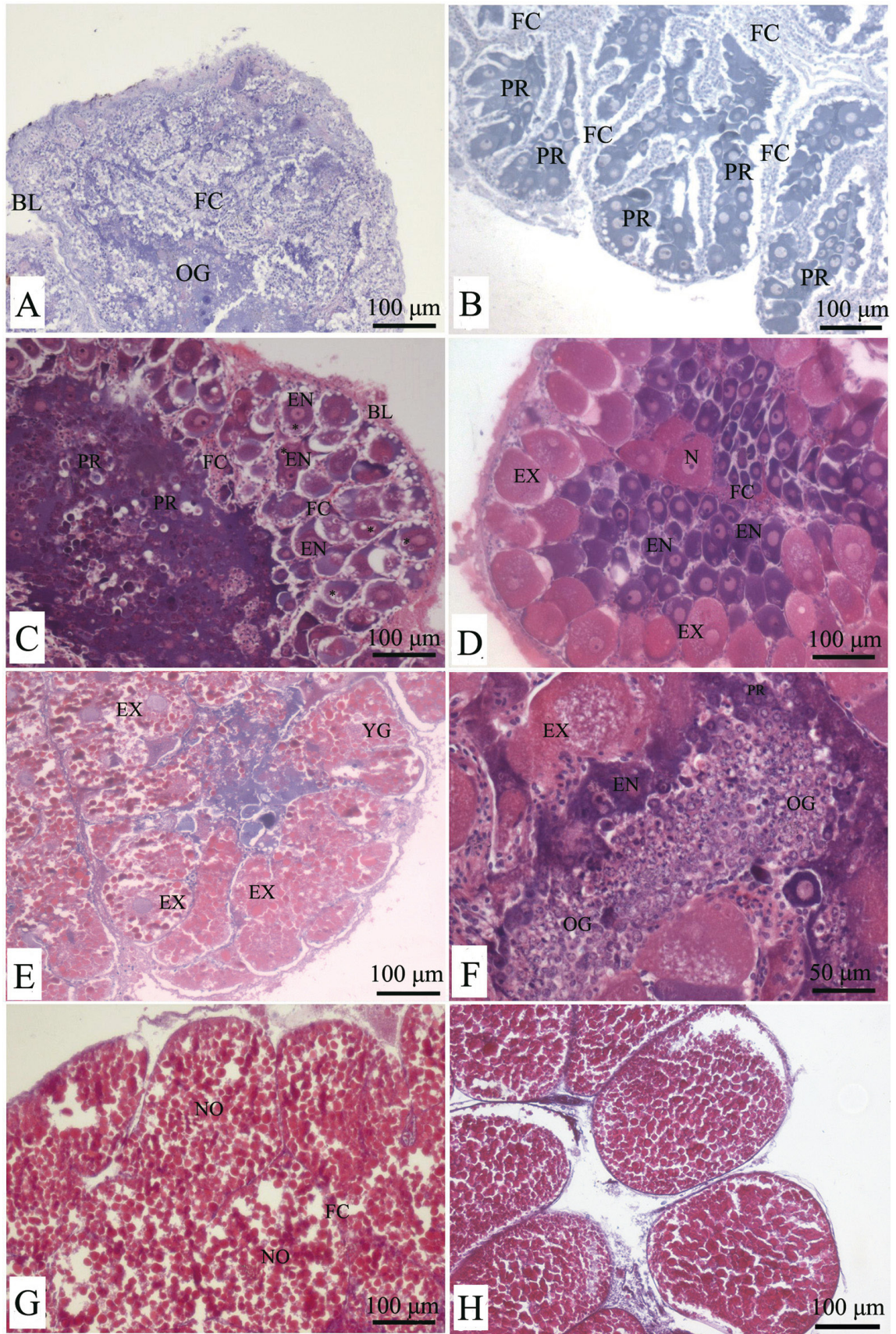


Fig. 4. – Ovarian histology of *Portunus pelagicus* during the reproductive cycle. A, early stage I ovary, showing oogonia (OG) and follicle cell (FC); B, late stage I ovary, showing previtellogenic oocytes (PR) and FC; C, stage II ovary, showing EN and small eosinophilic components (*); D, early stage III ovary, showing EN and EX; E, late stage III ovary, showing EX with YG; F, stage III ovary, still containing OG zone between the EX; G, late stage IV, showing NO; H, late stage V, showing MO.

Table 2. – The main features of different ovarian development stages during the ovarian maturation of *Portunus pelagicus*.

Ovarian stages	External ovarian features	Ovarian histological features	GSI(%)	Pubertal moult
I	Ovary is small and slightly convoluted, appears transparent, or translucent.	Oogonia and previtellogenic oocytes are the dominant types while they have not been surrounded by follicle cells. The cytoplasm is basophilic.	0.02-0.30	Most of them are before
II	Ovary increases in size and appears milky-white to off-white in colour. Two parallel ovary lobes are easily eye-visible.	Previtellogenic oocytes and endogenous vitellogenic oocyte appear dominant. Small yolk globules start to appear in exogenous vitellogenic oocytes and follicle cells have migrated to the periphery of the follicle.	0.27-0.54	Almost after
III	Ovary becomes light yellow or yellow. The ovary starts to cover parts of branchia and ovarian lobules become more apparent.	Exogenous vitellogenic oocytes are the dominant oocytes in ovarian lobules while the minority of endogenous vitellogenic oocytes are located in the central part of the follicle. Large yolk globules appear in cytoplasm at late stage III. Follicle cells are compressed and closely surrounding the oocytes.	0.38-4.54	Almost after
IV	Ovary shows yellow or bright orange colouration, middle region of ovary starts to cover part of the stomach.	Dominant oocytes are nearly mature oocytes. Small yolk globules become apparent in the entire cytoplasm and nucleus reduce in size and locate sideways.	4.50-9.21	After
V	Ovary is deep yellow to red-orange and reaches maximum size. The middle region of ovary covers the whole stomach. Ovarian lobules appear swollen and some mature oocytes become easily detached from the ovarian lobe.	Most oocytes are fully mature and have the homogenization of yolk globules, which are distributed over the entire cytoplasm.	8.31-14.51	After

Stage III: The ovary appear light yellow or yellow and its size increased drastically compared with the early ovarian stage. The anterior region of two parallel ovary lobes had a conspicuous M shape (Fig. 3E, 3F). The central region of lobes enveloped the dorsal surface of the spermathecae. In advanced stage III, the ovary started to cover the basilar parts of the branchia and ovarian lobules become more visible (Fig. 3F). The GSI of stage III ovaries increased dramatically from 0.38 to 4.54. The early stage III ovaries mainly contained EX (79.71%) and EN (20.28%). EX were localized in the periphery of each follicle (a small spherical group of cells) while EN were distributed in the central part of the follicle (Fig. 4D), where EX started to contain yolk granule (YG). The mean long diameter of EX was 107.28 μm for the early stage III ovary and in the later stage III ovary the dominant oocyte type was EX filled with YG (Fig. 4E). Occasionally, a few OG and PR still existed between EX (Fig. 4F). The mean long diameter of EX was 168.54 μm for the later stage III, significantly longer than that of early stage EX ($P < 0.05$).

Stage IV: The ovary showed yellow to bright orange colouration. Ovary lobes occupied most available body cavities and the mid-lobes covered part of the stomach (Fig. 3G). The GSI of stage IV ovaries ranged from 4.50 to 9.21 and histological studies showed that this stage was dominated by NO. The mean long diameter of NO was 258.37 μm , significantly longer than the EX of stage III ovaries ($P < 0.05$). In NO, the small YG became apparent in the entire cytoplasm of NO (Fig. 4G). The uniform presence of small YG was an indicator of NO development. The nucleocytoplasmic ratio of NO decreased more significantly than that of EX (Table 1) and the nucleus located peripherally was hardly recognizable in this stage (Fig. 4G). The follicle cells were not easily recognizable because of the crowding of the oocytes.

Stage V: Mature ovaries were deep yellow to red-orange in colour and reached the maximum size, with the middle lobes covering the whole stomach (Fig. 3H). The gastric, posterior and intestinal cavities were completely filled with the large central and posterior sections of the ovary. The ovarian lobules appeared swollen and some MO became easily detached from the ovarian lobe when handled (Fig. 4H). The histological sections showed the majority oocytes to be fully mature at this stage. The MO had the uniform YG, which were distributed evenly in the entire cytoplasm while the nucleus was small, located peripherally and not easy recognizable (Fig. 4H). In some cases, the MO were separated from the follicle cells, indicating that spawning was imminent (Fig. 4H).

Changes in GSI, HSI and oocyte diameter during ovarian development

During the ovarian maturation, the GSI increased with the ovarian development while the HSI was decreased (Table 3). The highest HSI was found in stage II. Within the same ovarian stage, the HSI varied significantly: e.g. the HSI ranged from 2.09 to 8.59 for stage I female crabs. Furthermore, a significantly negative correlation was found between GSI and HSI (Fig. 5, $n=52$, $P < 0.001$, $r = -0.484$). From ovarian stage III to V (exogenous vitellogenic phase), a significant positive correlation was found between GSI and long diameter of oocytes (Fig. 6, $n=32$, $P < 0.001$, $r = 0.956$).

Size at sexual maturity

In the present study, the minimum size at first maturity was 78.5 mm CW based on ovarian development (stage III) and the size of the smallest berried crab was 79.2 mm CW. However, about 60% of females in the

Table 3. – The gonadosomatic index (GSI) and heptosomatic index (HSI) at different stages of ovary mature in *Portunus pelagicus*. Values are presented as mean±SE. Different superscript letters within a same row indicate significant differences (P<0.05).

Ovarian stage	I	II	III	IV	V
Sample number	12	9	13	10	8
GSI (%)	0.18±0.02 ^a	0.39±0.04 ^b	1.90±0.34 ^c	7.91±0.40 ^d	11.50±0.75 ^e
Range of GSI	0.02-0.30	0.27-0.54	0.38-4.54	4.50-9.21	8.31-14.51
HSI (%)	5.56±0.32 ^{bc}	6.44±0.62 ^c	4.46±0.40 ^{ab}	4.06±0.36 ^a	3.70±0.50 ^a
Range of HSI	2.07-8.59	2.50-8.49	2.16-7.63	2.32-6.23	2.03-5.74

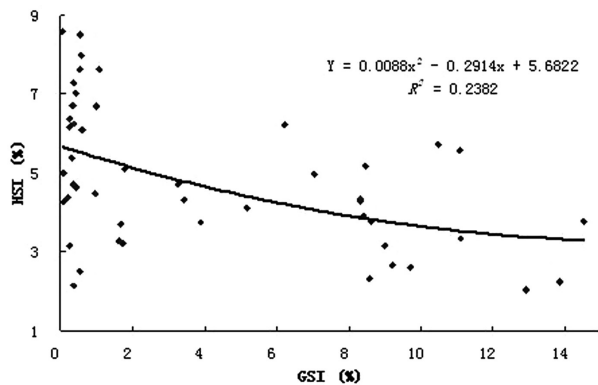


Fig. 5. – The correlation of gonadosomatic index (GSI) and heptosomatic index (HSI) during the ovarian development of *Portunus pelagicus*.

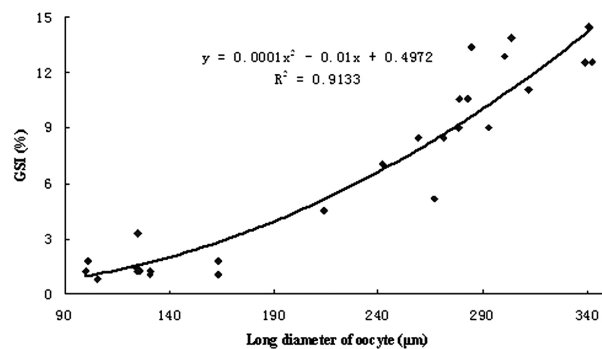


Fig. 6. – The correlation of mean long diameter of oocyte (µm) and gonadosomatic index (GSI) during ovarian stage III to V of *Portunus pelagicus*. Each female had its own GSI and mean long diameter of its oocyte, and these data were used for the correlated analysis.

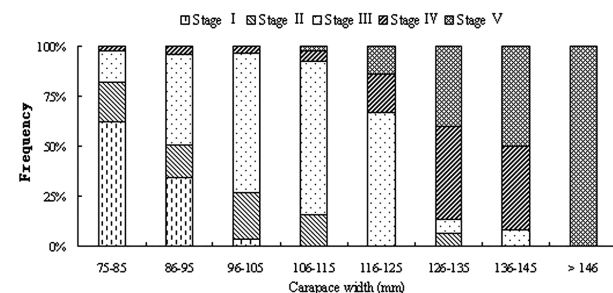


Fig. 7. – Frequency of occurrence of each ovarian developmental stage in different size classes of *Portunus pelagicus* in Beibu Gulf.

75-85 mm size class had not completed the pubertal moult (Fig. 7) and the ovarian staging of these females was stage I. All of the females with more than 106 mm CW had undergone pubertal moult during this study. The frequency of crabs with mature ovary (EX, stage III-V) increased as the body size increased. The frequency of occurrence of crabs with mature ovary

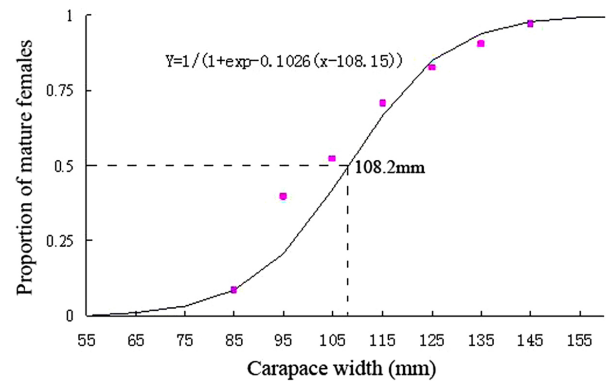


Fig. 8. – Logistic curve showing the proportion of mature *Portunus pelagicus* females in Beibu Gulf at each internal carapace width class (size class=10 mm).

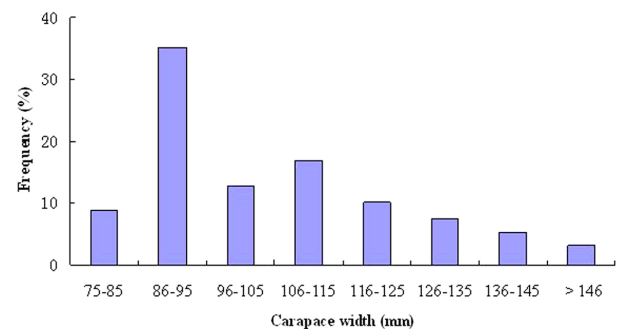


Fig. 9. – The frequency histograms for the carapace width of adult females of *Portunus pelagicus* in Beibu Gulf.

increased above 106 mm CW (≥84.6%) (Fig. 7). The CW at which 50% of females were mature (CW₅₀) was estimated to be 108.2 mm, while 95% of mature females were found to be mature at 136.9 mm (Fig. 8).

Size distribution

The size distribution of *P. pelagicus* in Beibu Gulf is given in Figures 9 and 10. The CW of adult females ranged from 78.5 to 162.0 mm, and the dominant size classes were 86-95, 106-115 and 96-105 mm, which included 64.9% of total adult females, while the proportion of females with >146 CW accounted for 3.1% only. BW of the crabs ranged from 32.5 to 372 g, and the dominant size class was 30-60 g, which accounted for 43.2%. In the majority of adult females BW was 40-120 g (71.4%), while BW>120 g or <40 g only accounted for 22.9% and 5.7%, respectively.

Monthly variation of the ovarian development and the percentage of ovigerous females

All the ovarian stages were represented in the samples in September-December except in October (Table

Table 4. – The percentage of different ovary development stages in *Portunus pelagicus* from Beibu Gulf.

Month	n	Stages of ovary				
		I	II	III	IV	V
Sep	30	13.3	20.0	50.0	10	6.7
Oct	30	6.7	33.3	53.3	6.7	0
Nov	30	23.3	33.3	33.3	6.7	3.3
Dec	32	9.4	25.0	50.0	12.5	3.1
Jan	50	0	20.0	56.0	20.0	4.0
Feb	59	0	5.1	41.0	27.9	23.7
Mar	40	0	0	22.5	50.0	27.5
Apr	34	0	0	41.2	47.0	11.8
May	31	0	16.1	58.1	19.3	6.5

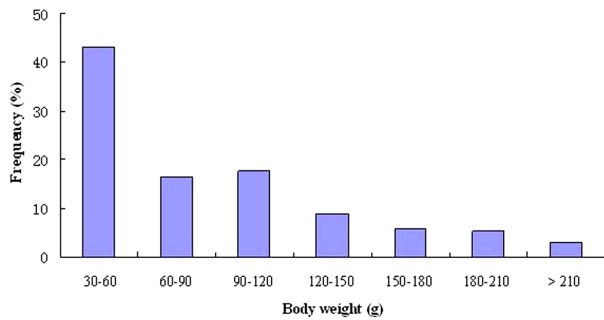


Fig. 10. – The frequency histograms for the body weight of adult females of *Portunus pelagicus* in Beibu Gulf.

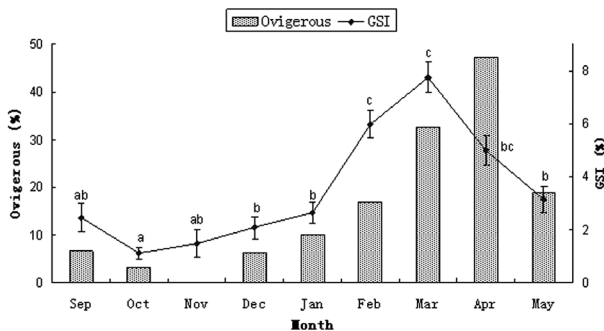


Fig. 11. – The monthly variation of the GSI and the percentage of ovigerous females of *Portunus pelagicus* in Beibu Gulf. The diamonds with different letters indicate significant differences between the months ($P < 0.05$).

4) and in the rest of the months the majorities were in stages III-V. The highest percentage of stage V ovary was recorded in February and March (23.7% and 27.5%, respectively). The mean GSI of adult females peaked at 7.74% in March, and the primary spawning period was found between January and May, as at least 10% of adult females were ovigerous during this period and their GSI varied from 0.58% to 9.01% (Fig. 11).

DISCUSSION

Ovarian development and staging

The cycle of ovary and oocyte development of *P. pelagicus* has been reported by several researchers (Bawab and El-Sherief 1988, Sheild and Wood 1993, Sumpton et al. 1994, Sukumaran and Neelakantan 1998, Stewart et al. 2007). However, their criteria of ovarian staging of *P. pelagicus* are quiet different. For example, Bawab and El-Sherief (1988) classified the

ovarian cycle of *P. pelagicus* into eight stages based on morphological changes of female spermatheca and copulation, while Stewart et al. (2007) divided the developing ovary into four stages based on histological studies and oogenesis. A reliable and feasible criterion for ovarian staging is necessary and important for the fishery management and hatchery seed production of *P. pelagicus*. This study is the first one to develop a comprehensive method for the ovarian staging of *P. pelagicus* by combining gonadosomatic index (GSI), pubertal moult, changes of external features and histology of ovaries. The results revealed that the females develop their ovaries via proliferation and differentiation of the oogonia prior to the pubertal moult, and this finding was supported by the fact that a few previtellogenic oocytes appeared in the late stage I. Similar findings have been reported for the other portunid species, i.e. the swimming crab *Portunus trituberculatus* (Wu et al. 2007), the mud crab *Scylla serrata* (Quinitio et al. 2007) and the mud crab *Scylla ovivacae* (Islam et al. 2010). The ovarian stage II is a transition from PR and EN to EX for *P. pelagicus*, and females with stage II ovary were generally post-pubertal moult. However, Stewart et al. (2007) divided the spawn-spent females into stage I of ovarian maturation of *P. pelagicus*, while Kumar et al. (2003) classified the ovigerous females into a separate stage. These results are inconsistent with our staging criterion. In newly spent females the dominant oocyte types were EN and PR rather than OG. Therefore, it is better to include the newly spent females in stage II. The ovarian morphology and histology of berried females of *P. pelagicus* revealed that they develop ovaries during the incubation period and the ovary stages varied between stages II-IV.

Normally, the ovary of post-puberty moult females is stage II or more advanced stages because copulation and deposition of sperms in the spermatheca can accelerate the ovarian development of portunid crabs (Yao et al. 2007). However, it was observed that from September to December there were still some females with stage I ovaries after the pubertal moult. This may be due to unsuccessful copulation in these females as soon as they completed the pubertal moult. Ovary colour progressively changed from transparent/translucent (stage I) to deep yellow or red-orange (stage V) during the course of their maturation because of the accumulation in the ovaries of carotenoids in the form of chromoproteins (Kalinina et al. 2008). However, the colour of the ovary is not strictly related to the developmental stage, particularly for the late stage I and stage

Table 5. – Comparative findings of the reproductive biology of female *Portunus pelagicus* from different locations around the world.

Location	Latitude, altitude	Minimum CW at maturity	CW at 50% maturity / mean CW	Peak of mating	Peak of mature crab	Ovigerous peak	Source
Carpentaria Gulf	16°25'S 139°44'E	60	72.2	---	July-Oct	Aug-Oct	Weng (1992)
Moreton Bay	27°20'S 153°10-20'E	79	106	Oct-May	Jul-Sep	Aug-Sep, Feb-Mar	Weng (1992); Shields and Wood(1993)
Shark Bay	25°40'S 113°41'E	61	92	---	No pattern	Jun-Aug, Dec-Jau	de Lestang et al. (2003b)
Peel-Harvey	32°39'S 115°40'E	61	97.5	---	Oct-Dec	Nov-Feb	de Lestang et al. (2003b) Potter et al. (1983)
Leschenault Estuary	33°15'S 115°42'E	84	98.0	---	Sep-Nov	Dec-Jau	de Lestang et al. (2003b)
Cochin	9°58'N 76°15'E	---	95	---	Dec-Mar	Aug-Apr	Pillai and Nair (1971)
Madras	13°05'N 80°18'E	---	96	---	Nov, Jan, Jun	Sep-May	Rahaman (1967)
Karnataka coast	12°57'-14°47'N 74°6-48'E	---	96	---	Dec-Feb	Aug-Sep, Feb-Mar	Dineshababu et al. (2008) Sukumaraan and Neelakantan (1998)
Beibu Gulf	19°58'N 109°45'E	78.5	108.2	Sep-Nov	Feb-May	Mar-Apr	Present study
Abu Kir Bay	31°18'N. 30°9'E	80	---	---	Apr-Sep	May, Sep	Bawab and El-sherief (1988)

II, and stages IV and V. In some cases in our study the female *P. pelagicus* had a similar ovary colour in early stages between late stage I and stage II, and in advanced stages IV and stage V. In categorizing ovarian stages, apart from external characteristics of the ovary (colour, size, shape, etc.), ovarian histology, gonadosomatic index (GSI) and pubertal moult must also be taken into consideration. Our findings have shown that the percentage of different types of germinal cells, oocyte diameter, ovarian morphology (size, colour and shape, etc.) and GSI are four important aspects that should be taken into account in classifying the ovarian stages of *P. pelagicus* and that will be useful as benchmarks in future studies.

GSI and HSI during ovarian maturation

From ovarian stage II to stage III, the HSI declined significantly and GSI increased by 3.9 times. During the fast ovarian development of *P. pelagicus* (stages II-III), the nutrient reserve in the hepatopancreas was used to support the developing ovary, which led to a decrease in HSI during this period. A similar phenomenon was reported in the Chinese mitten crab, *Eriocheir sinensis* (Yu et al. 2007), and the swimming crab, *Portunus trituberculatus* (Wu et al. 2007). The positive correlation of GSI and long oocyte diameters suggested that the oocyte diameter could be used to predict the female GSI based on regression analysis (Fig. 6). Ovarian staging through in vivo examination of ovary using a biopsy method (Quinitio et al. 2007) would help researchers to predict spawning without sacrificing the female crabs (Table 2). This is important for the future hatchery seed production and reproductive studies on *P. pelagicus*, as mature or nearly maturing females are very expensive in some Asian countries (Stewart et al. 2007, Wu et al. 2010).

Size at maturity and size distribution

The minimum size at the first maturity of female *P. pelagicus* in Beibu Gulf was 78.5 mm CW, which is in conformity with the studies carried out in different parts of the world. Generally, the minimum CW at the first maturity of *P. pelagicus* tends to be inversely related to water temperature (Weng 1992, De Lestang et al. 2003b). In tropical regions, the species mature at smaller sizes than in subtropical and temperate waters. The CW at 50% female *P. pelagicus* maturity in Beibu Gulf (108.2 mm) was close to that of the Moreton Bay population (106 mm), but higher than that of three Western Australian and Indian populations (Table 5). This difference reflects not only inter-regional differences in the reproductive biology of female *P. pelagicus* but also differences in fishing pressure, sampling gears, sampling locations and seasons, crab density, predation pressure and food availability of natural habitat (Hines 1989, Weng 1992, de Lestang et al. 2003b, Smith et al. 2004, Johnson et al. 2010).

Previous studies have shown that the minimum legal CW of captured crabs could be roughly based on CW₅₀ (Islam et al. 2010, Overton and Macintosh 2002). Johnson et al. (2010) suggested that, in order to protect spawning females, the minimum legal CW of females should be more than CW₅₀. As the size at first maturity of *P. pelagicus* is regional and area-specific, the minimum legal sizes of CW vary accordingly (Kangas 2000). For example, the minimum legal size of *P. pelagicus* is 127 mm CW (including lateral spines) for both commercial and recreational fishers in Western Australia, but it is 150 mm CW in Queensland, Australia, and the population of Moreton Bay was composed of approximately 8%-10% of total adult females up to 150 mm (Weng 1992). In the present study fewer than 2% of total adult females ≥ 150 mm CW were found in the Beibu Gulf population of *P. pelagicus*, whereas

from the same region Wang et al. (2001) reported that 10%-20% of total adult females had a CW \geq 150 mm. These findings give a clear indication that the Beibu Gulf population of *P. pelagicus* have undergone high fishing pressure during the past decade, leading to the decline of the proportion of large crabs (Huang 2009). At present no management plans are in place for the fishery of *P. pelagicus* in Beibu Gulf: any size of adult *P. pelagicus* can be harvested during the fishing season (August 16-May 15) and it is now essential to implement more feasible management regulations to protect spawner-recruitment in the region.

Seasonality of ovarian development and spawning

The seasonal pattern of *P. pelagicus* spawning is known to be highly variable between geographic locations (Table 5). Gonadosomatic index (GSI) is a good indicator for the ovarian development of crustaceans (Wu et al. 2007), and in the present study a maximum was recorded in March for the Beibu Gulf population of *P. pelagicus* females. In low-latitude regions, the GSI of female *P. pelagicus* peaked earlier than that in high latitude regions (Smith and Sumpton 1989, Sumpton et al. 1994). It is known that water temperature is considered as a controlling factor for ovarian development and spawning of *P. pelagicus* (Potter et al 1983, de Lestang et al. 2003b). Huang et al.(2008) reported that in winter (December to February) the seawater temperature for the present study area was 18-20°C (Huang et al. 2008). However, female *P. pelagicus* can spawn at 18°C but no eggs would hatch out at that temperature after 68 days of incubation (Campbell 1984). The peak of ovigerous females occurred in April for the Beibu Gulf population of *P. pelagicus* females, which may be due to the accumulation of their spawning from January to April. Weng (1992) and Sheild and Wood (1993) reported that after the copulation, the ovary of *P. pelagicus* develops fast. The stage II ovary is a very short phase after pubertal moult and mating, which actually are the transition phase from PR/EN to EX for the ovary, and ovarian stage II of *P. pelagicus* lasted only 2-7 days with water temperature between 26°C and 32°C with feeding (Wu et al. unpublished). Therefore, the high proportion of stage II females during the period September-December indicates that the first pubertal moult and mating may occur intensively during this period for females of less than 1 year. The highest number of spawners is found in February-May, but berried crabs are recorded throughout the year in Beibu Gulf.

CONCLUSION

Based on pubertal moult, GSI, morphology and histological studies, ovarian development in *P. pelagicus* can be divided into five stages. The differences in reproductive biology of female *P. pelagicus* indicate that these reproductive parameters are geography-specific. This is the first study to report the reproductive biology of female *P. pelagicus* from the Beibu Gulf population and the variation in reproductive performance of female *P. pelagicus* is specific to the geographical area.

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