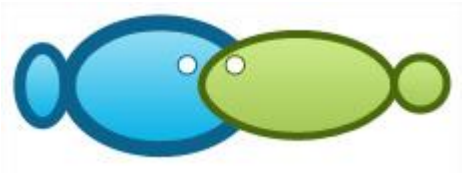

Study on the reproductive biology of the blue swimming crab, *Portunus pelagicus* females from Pattani coastal waters, Thailand

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Abstract. This study emphasizes on aspects of fecundity, embryonic development, ovarian development, maturity stage and correlation of matured blue swimming crab, *Portunus pelagicus* females according seasons and habitat in the lower part of the Gulf of Thailand of Pattani coastal water. Samples of matured *P. pelagicus* were collected by crab gill nets monthly from May 2013 to July 2014 in Pattani bay and bimonthly from May 2013 to September 2014 in offshore area. It was found that sizes (CW) and body weight (BW) of crab in this study were 8.71 to 13.52 cm CW and 48.04 to 235.04 g BW in Stage 1, 8.80 to 15.82 cm CW and 58.12 to 326.35 g BW in Stage 2, 9.71 to 16.34 cm CW and 62.19 to 428.15 g BW in Stage 3. Mean fecundity in Stages 1, 2 and 3 of the embryonic development stages were 432,119±131,922, 545,953±21,2857, 956,314±370,600 eggs respectively. Mean diameter of egg size (μm) for berried female embryonic development Stages 1, 2 and 3 were 0.298±0.018, 0.355±0.026, 0.358±0.014 respectively. Mean egg mass index of Stages 1, 2 and 3 were 0.20±0.04, 0.09±0.03 0.08±0.02 respectively. Mean oocyte diameter (μm) increased drastically from immature (Stage 1: 112.354±70.953), early maturing (Stage 2: 148.814±1.756), late maturing (Stage 3: 158.944±1.763) to fully mature (Stage 4: 178.146±3.665). It was found a significant relationship between fecundity and CW/BW of crab. Egg mass weight was increased proportionally with CW and BW in all embryonic stages. Egg mass index was decreased proportionally with CW and BW in all embryonic stages. Size at sexual maturity for female *P. pelagicus* was 9.97 cm CW. Moreover, different habitat of the Pattani coastal waters had a significant impact ($p<0.05$) on number of mature female in Pattani bay. As the conclusion, eggs mass were increased with CW and BW. Habitat also influenced matured female in Pattani coastal water.

Key Words: fecundity, ovarian maturation stages, size at sexual maturity, Pattani bay.

Introduction. Blue swimming crab, *Portunus pelagicus* is a scavenger species and very popular around the world as one of the target species for fishermen and recreational purposes. This causes the higher demand in the market by any size of *P. pelagicus*. The delicious meat and stable price of *P. pelagicus* had led to the overfishing of this species. Many countries around the world such as Malaysia (Ikhwanuddin et al 2012a; Ikhwanuddin et al 2014), Thailand (Islam & Kurokura 2012), Nigeria (Emmanuel 2008), India (Soundarapandian & Raja 2008) and Australia (Johnson 1980; Potter et al 1983; Xiao & Kumar 2004; Svane & Hooper 2004; Johnson et al 2010) are actively involved in both in research and fisheries development of *P. pelagicus* and other Portunid species.

Thailand is the fourteenth largest marine producer in the world, including crabs, with an annual production of about 1.6 million tons in 2012, worth approximately 1.29 million USD (FAO 2014). However, total marine landing of *P. pelagicus* fall 39.2% compared to 42.5% in 2011. This similar trend was also happened in Pattani coastal water, Thailand where landing has decreased 12% in 2012 (Thailand Fisheries Department, 2014). Although *P. pelagicus* is considered an important economic species, published information on reproductive biology is still lacking to be referred from Thailand

(Nitiratsuwan et al 2010, 2013). Most of the literatures are strictly limited to record of occurrence or report in unpublished literature. The work by Tantigul (1984) was considered one of the earliest studies on the fisheries biology of *P. pelagicus* in the Gulf of Thailand. Jindalikit et al (2004) studied the biology of *P. pelagicus* in the upper Gulf of Thailand. Furthermore, fishers are capturing all sizes of *P. pelagicus* without concerning any ecological impact and its management. The catch of small-sized crabs and ovigerous female, which are also the practices popular capture fishery practice in Thailand coastal water, will lead to a decrease of stock.

Therefore, an understanding of reproductive biology of this species is very important aspect in management especially in evaluating harvest strategies of exploited populations. This study is therefore conducted with the aims to determine the reproductive biology aspects which included fecundity, embryonic development, ovarian maturation and size of maturity of *P. pelagicus* female from Pattani coastal water, Thailand.

Material and Method

Study area description. Two different areas along Pattani coastal water, Thailand were selected for this study, Pattani Bay and Pattani offshore zone (Figure 1), where a majority of local fishers used crab gill net as main fishing gear.

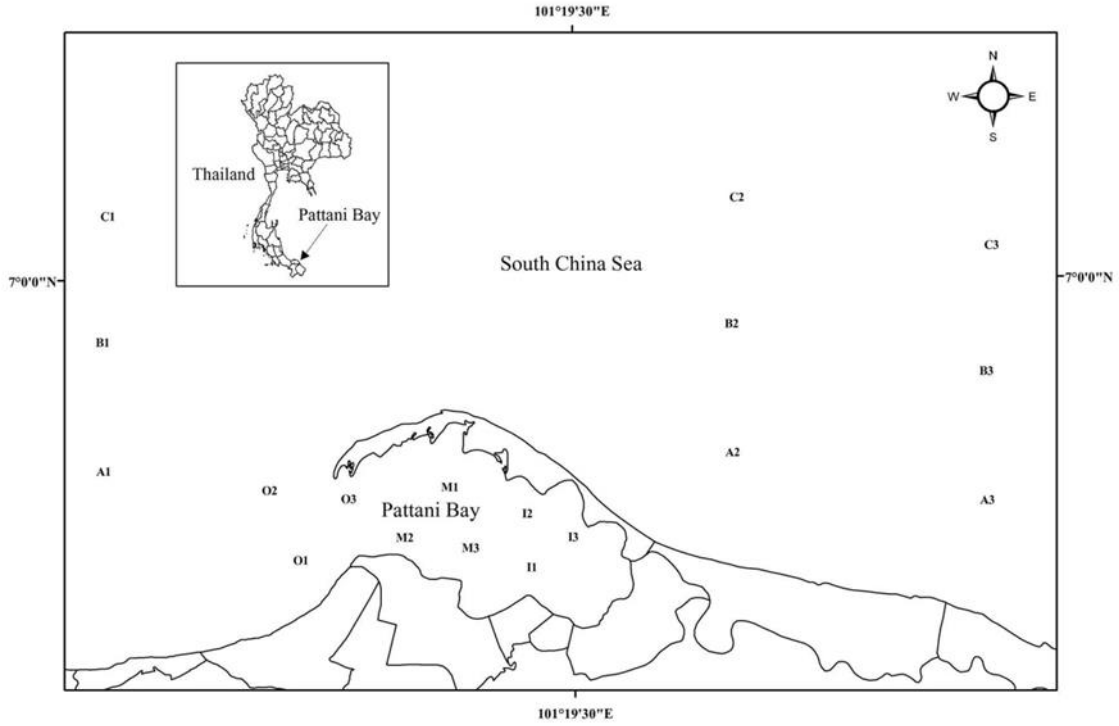


Figure 1. Map of study area showing the Pattani Bay with the three main habitats; Inner (I), Middle (M) and Outer (O) and Pattani offshore zone with three main contour water depth; A (5 m), B (10m) and C (15 m).

Pattani Bay is a 74 km^2 semi-enclosed estuarine bay protected on the northeast side by 12 km long sand spit. Areas of mangrove, both natural and managed (estimated at 900 ha), are found in the east of the area. The water regime is complex, with tidal influences from the Gulf of Thailand, run-off from the landward side and water drains from the two major rivers, Pattani and Yamu River. Mainly the water depth was between 0.2-1.5 m with the maximum of 5 m at the bay mouth and deeper gradually outside the bay. Mangrove forests dominate the surrounding areas of the bay consisting of *Rhizophora micronata*, *R. apiculata*, *Sonneratia alba*, *Avicennia officinalis*, *Bruguiera gymnorhiza*, *B. cylindrica*, *B. parviflora*, *Kylocarpus moluccensis*, *Acanthus ilicifolius*, *Excoecaria agallocha*

and *Nypa fruticans*. Generally the Pattani Bay is divided into three main habitats; inner, middle and outside bays. The inner bay characterized by 1.0-1.5 m deep with a muddy bottom and some coverage of seagrasses and seaweeds. The middle bay habitat is 1.0-2.0 m deep with a combination of sandy-muddy bottom supporting some *H. ovalis*, *H. beccarii, H. uninervis* and the algae *Ulva* spp. and *Gracilaria* spp. In the outer bay, it is characterized by 2.5-4.0 m deep with muddy bottom and without any vegetation. For Pattani offshore zone, an open water area with three different depths contours along coastal area were selected; 5 m, 10 m and 15 m depths.

Collection of samples. In Pattani bay, mature *P. pelagicus* female was monthly sampled from May 2013 to July 2014 by crab gill net. The gill net was 180 m long, 1.64 m deep with 8 cm stretch mesh size. The gear was set at 06:00 hours, left overnight for 24 hours and hauled on board in the next morning. Set of nine nets were placed covering all area at each three main habitat. Altogether, a total of 1,620 m long nets were used for each main habitat.

For offshore area, samples were sampled bimonthly from May 2013 to September 2014 using also the crab gill nets. The net dimension used are 1,800 m long, 1.54 m deep and 11 cm stretch mesh size. The fishing gear was set also at 06:00 hours, left overnight for 24 hours and hauled on board in the next morning. Altogether, 5,400 m long of nets were used at each depth contour and altogether 16,200 m, from all three depth contours were hauled for each sampling month. *P. pelagicus* were removed from nets and kept in iced during each sampling trip.

Mature female crab identification. Crabs are identified as juveniles and immature by an abdominal flap position attached firmly to the thorax (Van Engel 1958). Crab physiological maturity can be identified with the development of gonad, especially the enlargement of gonadal breeding system in male crab and the expansion of ovarian mass with visible color changes in female crab after maturity (Waiho et al 2017). Mature female crab characteristic is loose abdominal attached to the thorax and flap form to oval shape as it happened due to moulting process (De Lestang et al 2003b).

Carapace width (CW) and body weight (BW) measurements. In order to standardize wet body weight (BW), each of *P. pelagicus* specimens was placed in fresh water at least one minute, blotted dry for 30 seconds, and weighted after 30 seconds of air drying (Cadman & Weinstein 1985). For dried BW, crab was dried at 60°C for 48 hours after gonad and stomach extraction and labeled (Swiney & Shirley 2001). Only a perfect body of *P. pelagicus* was chosen for wet and dried BW. Carapace width (CW) was taken from the distance between the 9^{th} anterolateral spines of the carapace and recorded to the nearest 0.1 cm (Ikhwanuddin et al 2009).

Control experiment for embryonic color. Three alive berried female in each three different embryonic egg color stages; yellow orange color, brown color and black color sampled caught by the gill nets were selected randomly and transferred to laboratory. Three berried females were placed in the 1 m^3 capacity hatching tanks with maximum density of three crabs according to embryonic stage. Tanks were provided with 18 cm of thick sand and adequate aeration. The tank waters depth was 30 cm with salinity of 30-33 ppt, pH 7.00 to 8.00, temperature 26-27^{o}C, and oxygen 7.00-7.50 ppm. Shelter were made from PVC pipe to prevent from attack by others berried female and as rest place due to molting stage. Fresh mollusk, squid and prawn meats were given once in day at 19:00 h and uneaten food items were removed in next morning.

Fecundity. Three different embryonic egg color stages were identified by color as follows; a) Stage 1: yellow to orange, b) Stage 2: brown c) and Stage 3: black (Ikhwanuddin et al 2012b). The alive berried females were immediately killed in the laboratory by placed them into iced water for 5 minutes. Egg masses were carefully stripped off from the broad abdomen of crab pleopod and washed with fresh water. The eggs then were preserved in Modified Gilson's fluid (Ikhwanuddin et al 2011) for further

analysis. The chemicals used for the Gilson's fluid are as follows; 100 mL of 60% alcohol, 880 mL of water, 15 mL of 80% nitric acid, 18 mL of glacial acetic acid and 20 g of mercuric chloride. After that, the preserved egg mass was shaken vigorously and left for 24 h. After 24 h, the shaken activity was repeated to separate eggs from tissues and helped a penetration of preservative. The egg mass was preserved for the three months. After three months, the preserved egg mass was washed with distilled water before measure egg diameter (using ocular microscope), weight (micro balance of 30 eggs) and counting using volumetric methods (Ikhwanuddin et al 2012b).

For volumetric methods, all the washed eggs were placed into 200 mL distilled water. Water was slowly poured to ensure the eggs were completely spread and did not reside in one water column. The 1 mL syringe was used to take 1 mL of water from 200 mL of the mass eggs. The 1 mL egg from syringe was measured by using Nikon Measuring Microscope and egg number was counted by the Sedge-Wick Rafter cell. This process was repeated five times to obtain mean value and for fecundity estimation.

For egg weight calculation, five decimal places of accurate measurement (0.00001 g) were used with micro balance (Mettle Toledo). The egg mass index was measured according to Sukumaran & Neelakantan (1997) formula:

Egg weight / Body weight

For egg size, 100 eggs in each embryonic egg stage of stage's 1, 2 and 3 were measured using ocular microscope. Mean and standard deviation of egg size was recorded.

Ovarian maturation. Ovarian maturation stages were determined based on two criteria of external morphology and histology examinations which was studied previously by Quinitio et al (2007), Ikhwanuddin et al (2009, 2011, 2012a) and De Lestang et al (2003a).

Gonad Somatic Index (GSI). After BW was recorded, gonad tissue of female crab was extracted and weighted. For dry weight measurement, gonad from each crab was extracted and body and gonad were kept for 48 h at 60°C to obtain the dry weight (Corgos & Freire 2010). Five mature females of *P. pelagicus* were selected randomly in each class of 5 cm CW for maturity analysis.

The Gonadal Somatic Index was calculated according to the formula:

$$GSI = \frac{GW}{BW} \times 100$$

Where:
GW: Gonad weight
BW: Body weight

Histology procedure for ovary examination. Twenty-four samples of crabs in each ovarian maturation stage of female were randomly selected. Ovary was fixed in the Boin's solution for 12 h (Johnson 1980; Ikhwanuddin et al 2012a). Small portion of anterior, middle and posterior ovarian were taken for further analysis. Then, tissues were dehydrated through different concentration of alcohol. The concentration are as follow; 12 h of 70% alcohol, 2 h of 95% alcohol, 2 h of 95% alcohol, 2 h of 100% alcohol, 2 h of 100% alcohol, xylene 1 h of xylene two times, 1 h of wax and finally 1 h of mix solution (50% wax : 50% xylene) (Roberts 2001; Ravi et al 2013).

Tissue processing was performed in the Automatic Tissue Processor Machine, called Jung Histokinette. After processing and hydration of tissues, wax impregnation was done through embedding process in a paraffin wax to form a solid block. Solid block was later cut into 5 µm sections and put in water bath for a floating process to stretch up sample. Samples were stained with haematoxylin and eosin (Roberts 2001; Ikhwanuddin et al 2012a). All the microscopic images were recorded by Dino-capture 2.0 software.

Statistical analysis. Means ± Standard Deviation (SD) for fecundity, body weight (BW), carapace width (CW) and oocyte diameter of the ovary histology examination were calculated.

Linear regression and correlation analyses were used to detect relationship between fecundity and BW and fecundity and CW.

A two-way analysis of variance (ANOVA) was used to compare; (1) abundance of mature female *P. pelagicus* between the sampling habitats and seasons. Numbers of mature female were log (X+1) transformed to reduce non-normality prior to analysis.

Ratio of mature individuals determined by macroscopic observation in each size classes was fitted to sigmoid curve (referred to formula shown below).

$$P_{cw=} \frac{1}{1+e^{(S1-S2cw)}}$$ (Koolkalya et al 2006)

Where:
$P_{cw=}$ proportion of mature to immature crabs in each CW class (1 cm interval), and $S1$ and $S2cw$ are the equation coefficients.

Results. In general, 649 mature female crabs were dissected. The sample was 6.0 to 17.9 cm CW and 24.51 to 370.79 g BW (Table 1). Size and weight of fully mature female crab (Stage 4) in this study were 13.07±1.76 cm CW and 163.52±69.14 g BW respectively (Table 2). Details of female *P. pelagicus* and its GSI of wet and dried weight are presented in Tables 1 & 2. Details of some biological aspects of female *P. pelagicus* in Pattani coastal waters based on gonadal stage are presented in Table 2.

Table 1

Summary of body weight, gonad weight, gonad somatic index (GSI) of female *Portunus pelagicus* in Pattani coastal waters

CW (cm)	*Body wet weight (g) (mean±SD)*	*Body dried weight (g) (mean±SD)*	*Gonad wet weight (g) (mean±SD)*	*Gonad dried weight (g) (mean±SD)*	*GSI (wet)*	*GSI (dried)*	*N*
6.0-6.9	24.51±10.45	8.05±2.81	0.94±0.56	0.20±0.14	3.93±0.51	2.43±0.87	13
7.0–7.9	33.28±19.65	11.99 ± 10.21	1.66±1.33	0.46±0.46	7.19±6.40	4.31±4.55	24
8.0-8.9	39.02±7.83	13.98±3.60	2.33±1.89	0.55±0.31	6.16±3.30	3.98±2.22	62
9.0–9.9	51.74±9.14	15.06±4.36	2.97±1.57	0.73±0.46	7.80±10.60	5.37±4.89	77
10.0-10.9	72.41±12.90	23.61±5.09	4.52±2.34	1.22±0.80	6.97±3.68	5.21±3.23	117
11.0–11.9	95.17±17.60	30.27±6.51	7.69±4.54	2.07±1.20	8.24±5.32	6.89±4.05	88
12.0–12.9	132.0±19.41	41.7±7.00	10.3±7.69	2.52±1.61	7.86±6.09	6.04±3.75	94
13.0–13.9	164.35±33.80	52.10±8.92	12.35±7.61	3.28±7.61	7.51±4.56	6.22±3.35	83
14.0–14.9	212.47±28.00	67.7±10.28	22.68±19.48	4.95±2.30	10.26±7.66	7.28±3.22	46
15.0–15.9	242.54±31.70	77.74±9.87	20.55±16.74	4.91±2.12	8.33±5.72	6.38±2.78	23
16.0–16.9	300.96±38.40	99.22±14.91	37.80±36.19	5.54±2.58	12.17±11.05	5.62±2.74	18
17.0-17.9	370.79±28.9	121.28±12.18	47.58±42.20	7.03±2.53	13.27±12.30	5.9±2.37	6
			Total				655

N - number of crab examined.

Table 2

Summary of some biological aspects and ovarian development of female *Portunus pelagicus* in Pattani coastal waters based on gonadal stage

Gonadal stages	*CW (cm) (mean±SD)*	*Wet BW (g) (mean±SD)*	*Dry BW (g) (mean±SD)*	*N*	*Oocyte diameter (µm) (mean±SD)*
1	10.34±2.18	80.13±54.20	25.64±17.53	206	112.354±70.953
2	10.74±2.07	93.03±61.46	29.69±19.60	196	148.814±1.756
3	12.47±1.98	144.82±74.18	46.62±25.40	139	158.944±1.763
4	13.07±1.76	163.52±69.14	52.19±22.29	114	178.146±3.665

CW - carapace width, BW - body weight, N - number of crab examined

Embryonic development. A total of 120 mature female crabs were dissected to observe morphology of embryonic development. Details of results according to different egg stages of *P. pelagicus* in Pattani coastal waters are presented in Table 3.

Table 3

Details of embryonic development and fecundity according to different egg stages of *Portunus pelagicus* in Pattani coastal waters

	CW (cm)	*CL*	*BW (g)*	*Egg mass weight (g)*	*Egg mass index*	*Weight of 30 eggs (g)*	*Weight of each egg (g)*	*Fecundity (eggs)*	*Egg size (μm)*
Stage 1 (Yellowish-orange)									
max	13.52	6.93	235.04	36.11	0.29	1.563×10^{-3}	5.21×10^{-5}	805,320	0.323
min	8.71	4.03	48.04	9.38	0.13	1.112×10^{-3}	3.71×10^{-5}	222,920	0.275
mean	10.78	5.12	98.33	18.59	0.20	1.295×10^{-3}	4.32×10^{-5}	432,119	0.298
SD	1.38	0.80	44.98	5.62	0.04	8.6×10^{-5}	2.9×10^{-6}	131,922	0.018
Stage 2 (Brown)									
max	15.82	7.22	326.35	23.20	0.15	6.92×10^{-4}	2.31×10^{-5}	1,175,160	0.424
min	8.80	4.10	58.12	5.97	0.04	5.04×10^{-4}	1.68×10^{-5}	261,680	0.328
mean	11.52	5.42	134.44	11.14	0.09	6.19×10^{-4}	2.06×10^{-5}	545,953	0.355
SD	2.02	0.90	74.41	4.18	0.03	5.5×10^{-5}	1.8×10^{-6}	212,857	0.026
Stage 3 (Black)									
max	16.34	8.15	428.15	31.30	0.13	5.60×10^{-4}	1.87×10^{-5}	1,802,040	0.425
min	9.71	4.27	62.19	5.49	0.04	3.21×10^{-4}	1.07×10^{-5}	413,120	0.325
mean	13.05	6.34	199.88	14.08	0.08	4.41×10^{-4}	1.47×10^{-5}	956,314	0.358
SD	2.00	1.01	90.55	5.96	0.02	6.1×10^{-5}	2.0×10^{-6}	370,600	0.014

Histological characteristic of ovarian maturation stage. Histological characteristic of ovary for each maturation stage are descripted as follows (Figure 2):

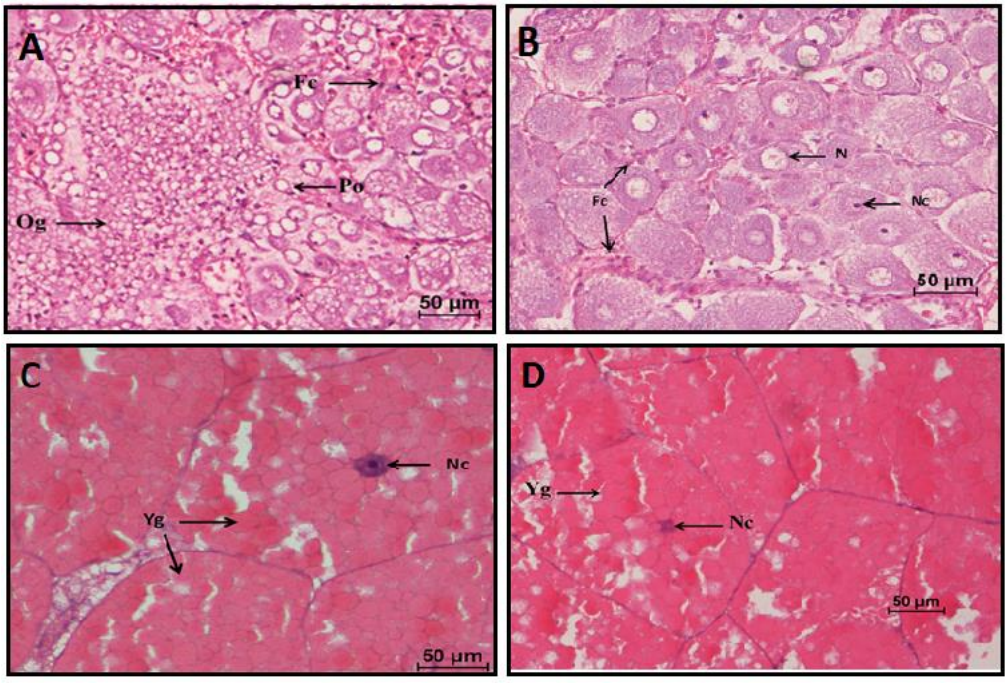


Figure 2. Histological result of ovarian development of *Portunus pelagicus* in Pattani coastal area. (A) Immature = Stage 1, (B) Early maturing = Stage 2, (C) Late maturing = Stage 3 and (D) Fully mature = Stage 4; oogonia (Og), follicle cells (Fc), primary oocytes (Po), yolk globules (Yg), nucleolus (Nc) and nucleus (N) (original).

Stage 1: Immature stage

Oocyte was transparent and translucent and difficult to determine. The follicle cells in this stage were almost in round shape and yolk globule did not exist. There was a specific

rectangle shape inside oocyte. Primary oocyte position was near the wall and small in size. The mean size of the oocyte in this stage was 112.354±70.953 µm.

Stage 2: Early mature

Oocyte was yellowish and yolk globule started to appear inside the follicle cells. The rectangle shape in this shape still appeared but some shrinking. Primary oocyte began to move closer to each other and bigger in size compared to stage 1. Mean size of oocyte in this stage was 148.814±1.756 µm.

Stage 3: Pre-mature

Oocyte was light orange in color. The specific rectangle shape inside oocyte almost disappeared. All follicle cells did not form a round shape inside primary oocyte. The follicle cells were easily recognized microscopically as oocyte grew rapidly and nucleus reached maximum size. Mean size of oocyte in this stage was 158.944±1.763 µm.

Stage 4: Fully Mature

Oocyte was deep orange in color and covered the hepatopancreas. Nucleus was appeared on this stage. The rectangular shape and primary oocyte inside the follicle cells disappeared in this stage. Mean size of oocyte was 178.146±3.665 µm.

External morphological characteristic of ovarian maturation stage. Total of twelve berried female *P. pelagicus* and three in each ovarian maturation stages were observed to determine duration of external color changing. Duration for ovary completed the maturation were eight days for dark yellow to orange, seven days from orange to brown and three days from brown to black. External morphological characteristic of ovary at each ovarian maturation stage are as follows (Figure 3):

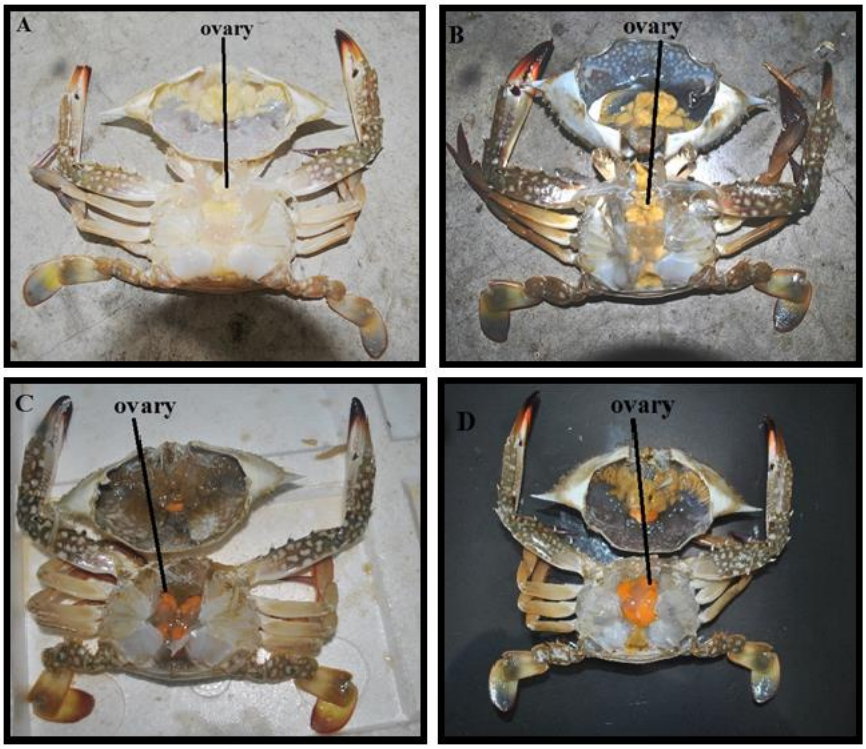


Figure 3. External morphological characteristic of *Portunus pelagicus* ovarian maturation stages; (A) Immature = Stage 1, (B) Early mature = Stage 2, (C) Pre-mature = Stage 3 and (D) Fully mature = Stage 4 (original).

Stage 1:

The ovary color was white and not fully developed, usually form "m" shaped. Ovary was translucent and hardly recognized from other tissues. Ovary was usually attached firmly with carapace.

Stage 2:
Ovary in right and left posterior was started to develop. Gonad color was milky to yellowish, and formed "H" shape as the size increase. Ovary can be recognized and separated from other tissues.
Stage 3:
Ovary was fully formed "H" shape with light and some dark orange colors. The ovary size increased larger.
Stage 4:
Ovary was fully in "H" shape with dark orange to light reddish colors. Ovary increased in size and eggs was visible by naked eyes. Ovary was thick in the posterior, anterior and middle surrounding other organ such as stomach.

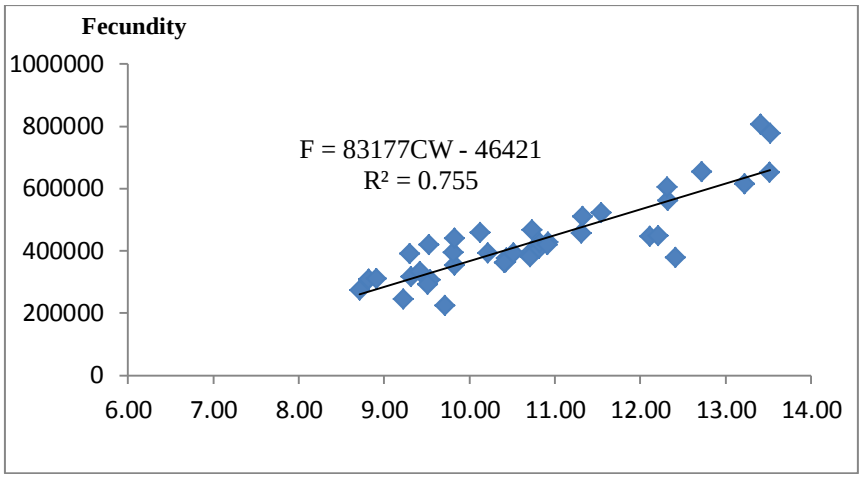
Fecundity and embryonic development. Three different stages of embryonic development were separated in this present study as in Figure 4. The details of fecundity for each embryonic development stage were given in Table 3. The result shows that, fecundity is significantly correlated with body weight and carapace width in all stages of *P. pelagicus*. Crabs with the same size classes but different embryonic stages showed different count of fecundity.

Figure 4. Embryonic development of *Portunus pelagicus* in Pattani coastal area based on color; (A) yellow-orange color = Stage 1, (B) brown color = Stage 2 and (C) black color = Stage 3 (original).

Relationship between carapace width and body weight with fecundity. Regression analysis showed that there was a highly significant relationship between number of berried eggs and carapace width in all stages of berried female ($p<0.001$) (Figure 5). The strongest correlation was in Stage 3.

Regression analysis showed that there was a highly significant relationship between number of berried eggs and body weight all stages of berried female ($p<0.001$) (Figure 6). The intensity of correlation was almost similar at all three stages.

(a)



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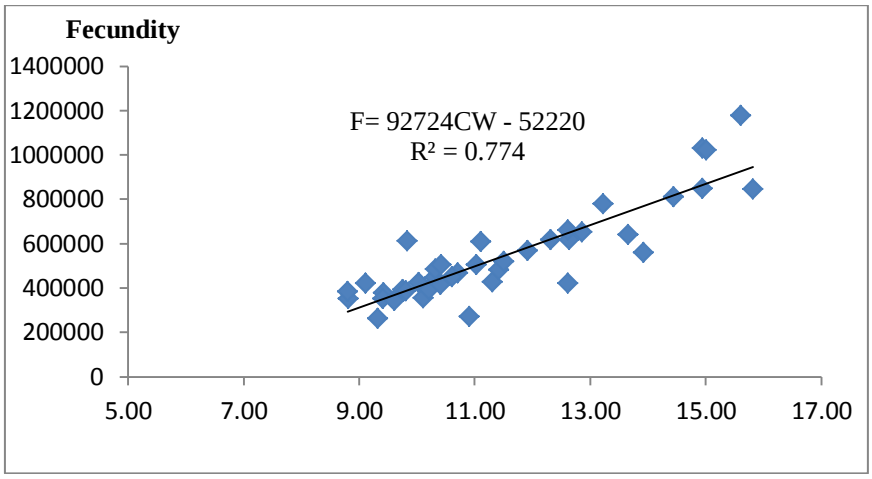

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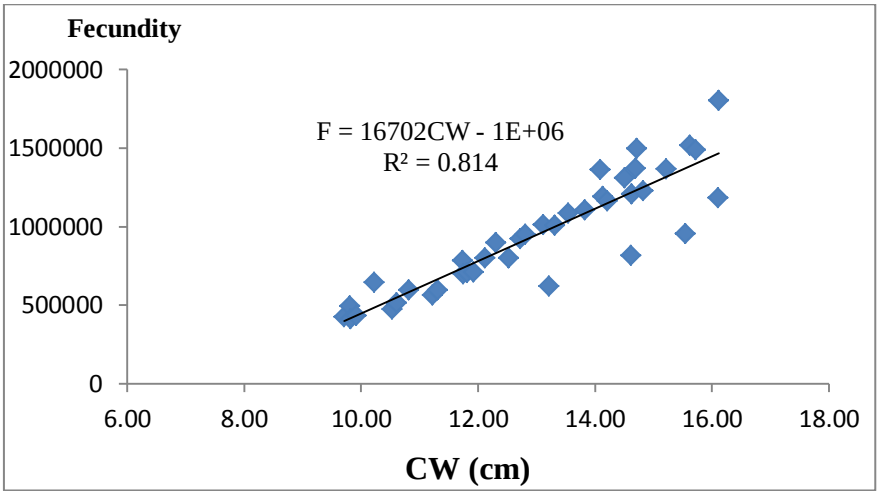
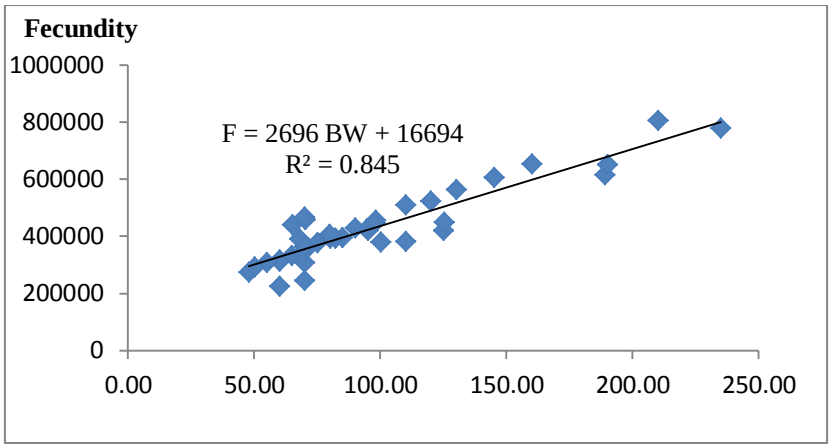


Figure 5. Relationship between carapace width (CW) and fecundity (eggs) for all three stages of berried female *Portunus pelagicus*; (a) = Stage 1, (b) = Stage 2 and (c) = Stage 3.

(a)



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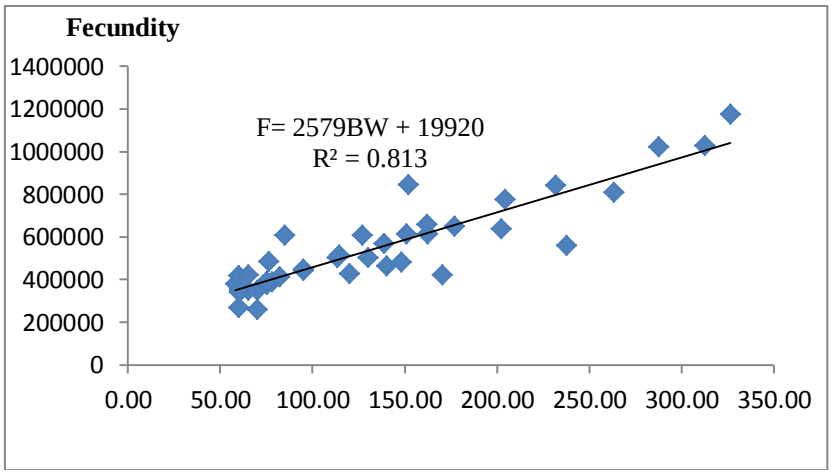

(c)

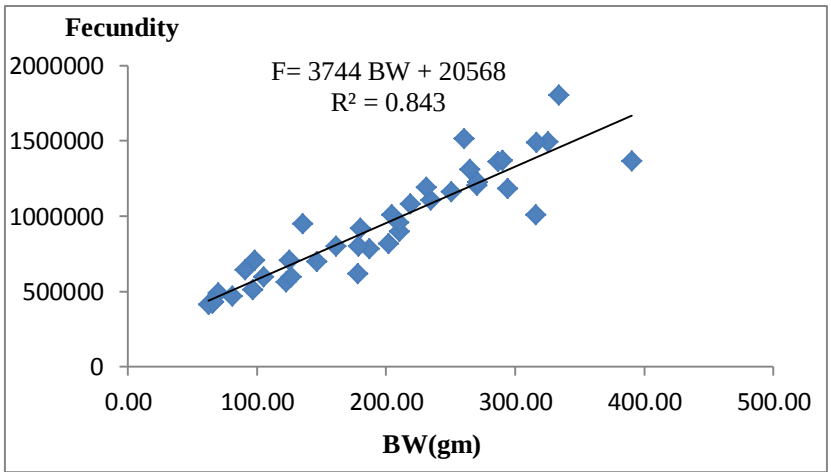

Figure 6. Relationship between body weight (BW) and fecundity for all three stages of berried female *Portunus pelagicus*; (a) = Stage 1, (b) = Stage 2 and (c) = Stage 3.

Size at maturity. Estimation of size at 50% maturity, a proportion analysis of CW indicated that the smallest and largest mature female in this present study were 6.00 and 18.00 cm CW, respectively (Table 4). In general, 50% of female *P. pelagicus* was estimated to reach sexual maturity at 9.97 cm CW (Figure 7).

Table 4

Carapace width (cm) frequency, percentage frequency, maturity frequency and percentage of mature female *Portunus pelagicus* based on ovarian maturation stage

Size range CW(cm)	*Frequency*	*Maturity frequency*	*Maturity proportion*
6.00–6.49	5	0	8.53×10^{-7}
6.50–6.99	5	0	4.95×10^{-6}
7.00–7.49	5	0	2.88×10^{-5}
7.50–7.99	5	0	1.67×10^{4}
8.00–8.49	5	0	9.72×10^{4}
8.50–8.99	5	0	5.62×10^{3}
9.00–9.49	5	0	0.03
9.50–9.99	5	1	0.16
10.00–10.49	5	1	0.52
10.50–10.99	5	1	0.86
11. 00–11.49	5	2	0.97
11.49–11.99	5	3	0.99
12.00–12.49	5	2	0.99
12.50–12.99	5	4	0.99
13.00–13.49	5	3	0.99
13.50–13.99	5	2	0.99
14.00–14.49	5	3	0.99
14.50–14.99	5	3	0.99
15.00–15.49	5	1	0.99
15.50–15.99	5	3	0.99
16.00–16.49	5	2	0.99
16.50–16.99	5	2	1
17.00–17.49	5	2	1
17.5–18.00	5	2	1
Total	120	37	-

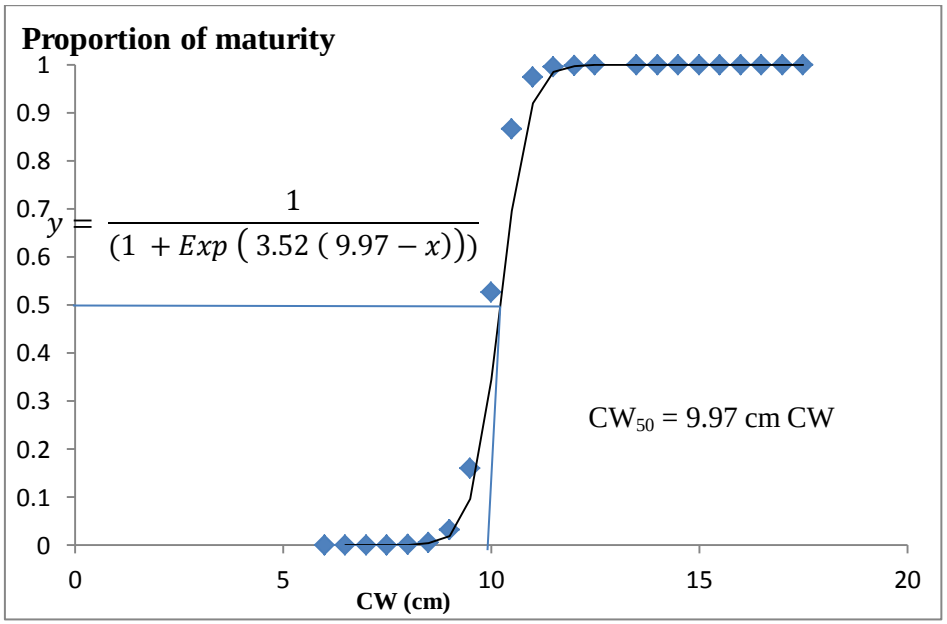

Figure 7. The proportion maturity log of female *Portunus pelagicus* to determine the size at sexual maturity (CW_{50}).

Impact of habitat and seasons. Result from analysis of variance (ANOVA) indicated that habitat, season and interaction between habitat and season had no impact on abundance of mature female in offshore area (P>0.05). However, a significant difference

was observed for abundance of female crabs found in different habitats from Pattani bay ($P<0.001$) regardless of season (Table 5).

Table 5

Results of two-way analysis of variance on effects of habitat and seasons on abundance of matured female crab *Portunus pelagicus* collected at two different areas along Pattani coast

Habitat	*Sources*	*df*	*MS*	*P value*
Pattani Bay	habitat (h)	2	2.430	9.27×10^{-5}
	season (s)	2	0.214	0.324
	h x s	4	0.378	0.111
Offshore	habitat (h)	2	0.107	0.156
	season (s)	2	0.025	0.610
	h x s	4	0.060	0.346

Discussion. Some aspects of the important biological information of *P. pelagicus* were found during the study period. It was found that mean diameter of eggs for each stage positively increased in accordance to the stages. They were 0.298 µm, 0.355 µm and 0.358 µm for stages 1, 2 and 3, respectively. Shape of the eggs in each stage was translucent and spherical similar to those found by Ikhwanuddin et al (2012b) and Robertson & Kruger (1994). Generally, it is known that older eggs have bigger size of mean diameter compared to the earlier stage. Each stage of egg colors can be an indicator of a maturity of the embryo. Three stages of embryonic development based on colors were recorded; Stage 1 (yellowish to orange), Stage 2 (brown) and Stage 3 (black). Duration for embryonic completed the maturation were eight days for dark yellow to orange, seven days from orange to brown and three days from brown to black. This finding was in contrast with those reported by Arshad et al (2006). Furthermore, this study also confirmed a highly significant relationship between carapace width and body weight with number of eggs at all stages.

Generally, two groups of findings for morphological characteristics of gonad development were divided. The first group indicated five stages of appearance (Johnson et al 2010; Sukumaran & Neelakantan 1997), and the second group divided into four stages (Ikhwanuddin et al 2012a, 2011, 2009; Quinitio et al 2007; De Lestang et al 2003a). Result from this present study can be divided into four histological characteristics which is similar to that of *P. pelagicus* in Australia (De Lestang et al 2003b) and *Scylla serrata* (Cheng et al 2002). It includes (1) formation of oocytic yolk associated with pinocytotic activity and follicle cells moved in surface of oocyte and surrounded the membranes, (2) intercellular space of oocyte appeared and ooplasmic membrane fused with follicular cell, (3) no membrane structure between the follicular cells and subjacent oocyte. Follicle cells directly moved to oocyte and stored as yolk bodies and lipid droplets and (4) two distinct layer forms by follicular cells and oocyte accumulated with irregular yolk bodies and oocyte sizes also increased (Cheng et al 2002).

It was found in this study that oocyte diameters for immature, early mature, pre-mature and fully mature were 112.354±70.953 µm, 148.814±1.756 µm, 158.944±1.763 µm and 178.146±3.665 µm, respectively. These figures were almost similar with a previous study for *P. pelagicus* in Johor, Malaysian coastal water (Ikhwanuddin et al 2012a). It is therefore able to conclude that diameter of oocyte of *P. pelagicus* and other portunid species will increase proportionally with gonad stages (Quinitio et al 2007; Ikhwanuddin et al 2012a). According to the present results, it is recommended that a combination of external morphology and histology examinations observations will be applied to determine ovarian maturation for *P. pelagicus* in order to achieve an accurate result. For example, it was found that some gonads were determined morphologically in Stage 2, but microscopic observation observed both Stages 2 and 3 of oocytes appeared in the same gonads particularly in posterior and anterior lobes. However, it is also suggested that to conserve time and cost of determination operation, external morphology technique based on ovarian color and characteristics is useful.

Fecundity can be defined as reproductive output produced from each batch of berried female (Arshad et al 2006) and berried female can have multiple batches in one season (Kumar 2000). It is proven by this study that fecundity is significantly correlated with body weight and carapace width in all stages of *P. pelagicus*. Crabs with the same size classes but different embryonic stages showed different count of fecundity. Compared to other studies in the region, result from this study was among the largest numbers of eggs counted from each crab of the same size classes. Egg numbers reached 1,000, 000 eggs for sizes ranging 14 to 17 cm CW. Ikhwanuddin et al (2012a) found that 13.32 cm CW and 235g BW of *P. pelagicus* had only 183,100 eggs. Arshad et al (2006) reported that a 14.58 cm CW, 268.3 g BW had 835,401 eggs. Kumar (2000) found that crab of >12.5 cm CW and 420.1 g BW had 761,432 eggs. The largest fecundity was reported by Johnson et al (2010) who found that crab of 75 to 79 mm carapace length and 333.70 g BW had 1,579,011 eggs. The larger number of fecundity ranging from 222,920–1,802,040 eggs, considered among the highest in the region, may cause by several factors. Ecological factors may influence a production of eggs at different study sites. Moreover, handling process during landing and incubation period may also be another very important factor. In this study, all berried females were caught by experimental crab gill net in the wild stock. All specimens were carefully released. Both of chelar propodus were tied with rubber band to limit movement of *P. pelagicus*. In order to reduce egg loss, live caught berried female was immediately killed in laboratory by ice. For crab of the same stage, it was found that the larger the crab the higher is the number of the eggs counted. This is supported by Brante et al (2003), Arshad et al (2006), Ikhwanuddin et al (2012a), Johnson et al (2010), Svane & Hooper (2004) and Kumar et al (2003) who found that smaller carapace width would produce low number of eggs.

Egg mass index was decreased proportionally with carapace width and body weight in all embryonic stages, whereas egg mass weight was increased proportionally with carapace width and body weight in all embryonic stages. It is proven by the present study that egg mass index and egg mass weight of *P. pelagicus* were correlated with body weight and carapace width. This finding also supports result from those found in India by Sukumaran & Neelakantan (1997).

A maturity of female *P. pelagicus* in this study was observed at 9.97 cm CW. It is considered a normal size for crab in the region as they are 9.50 cm CW in Malaysia (Ikhwanuddin et al 2009) and 8.90 cm in Pakistan (Rasheed & Mustaquim 2010). However a bigger size of maturity was reported in Australia at 12.7 CW (Potter et al 1983). Additionally, this study also found that in a semi-enclosed coastal bay, different sub-habitats significantly affected number of mature female. Inner bay and middle bay harbored small number of mature female crab compared to outer bay. This trend of impact was not detected in coastal area. Seasonal factors had no impact on abundance of mature female crab although some seasonal trend was observed.

As the conclusion, eggs mass were increased with CW and BW. Habitat also influenced matured female in Pattani coastal water.

Conclusions. As the conclusion, eggs mass were increased with CW and BW of *P. pelagicus*. It was also found that habitat influenced matured female in Pattani coastal water.

Acknowledgements. This research was financially supported by Faculty and Science Technology, Prince of Songkla University. The authors would like to express their deep gratitude to Malaysia Terengganu University for the scholarship, Pun Yeesin, Arun Loh-hem, Abdulroseh Cheloh, Sakariya Samae for the help of field and lab works. Many thanks also to the crew of fishermen for their assistance in field collection.

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Received: 08 June 2018. Accepted: 09 November 2018. Published online: 16 November 2018.
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How to cite this article:
Hisam F., Hajisamae S., Ikhwanuddin M., Aziz N. A. N., Naimullah M., Hassan M., 2018 Study on the reproductive biology of the blue swimming crab, *Portunus pelagicus* females from Pattani coastal waters, Thailand. AACL Bioflux 11(6):1776-1791.