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*Original Article*

# Reproductive performance and larval quality of blue swimming crab (*Portunus pelagicus*) broodstock, fed with different feeds

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**Abstract**

Fecundity, hatching rate and crab larvae quality of blue swimming crab, *Portunus pelagicus*, broodstock reared in earthen ponds, fed with different feeds, were studied for about 150 days. Results showed that average fecundity of *P. pelagicus* broodstock fed with trash fish (569,842±243,173 eggs), shrimp feed (464,098±188,884 eggs), mixed feeds (544,875.3±169,357.3 eggs) and hatching rate (50.78±25.19%, 62.33±26.79% and 50.86±20.76%) were not significantly different ($P>0.05$). Zoea produced by female broodstock fed with mixed feeds had a significantly higher survival rate (89.47±5.03%, $P<0.05$) compared to those produced by female broodstock fed with trash fish (73.40±14.61%) and shrimp feeds (67.33±12.80%), but the survival rate of megalopa and first crab stages was not affected. This study recommends that the mixed feed was suitable feed for rearing of *P. pelagicus* broodstock in an earthen pond as evidenced by the quality of zoea I or 1 day after hatching and growth of female broodstock.

**Keywords:** *Portunus pelagicus*, broodstock, reproduction, different feeds

## 1. Introduction

The blue swimming crab (*Portunus pelagicus*, Linnaeus 1758), a commercially important species, is distributed throughout the coastal waters of the tropical regions of the western Indian Ocean and the Eastern Pacific (Xiao and Kumar, 2004). Export of pasteurized *P. pelagicus* meat to the United States, Japan and Singapore generates multi-million dollar annual revenues for Indonesia. To meet the increasing market demands for soft-shell crabs (*P. pelagicus*), crabs have been individually held in compartments within a recirculating system to produce soft-shell crabs in Australia (Romano and Zeng, 2006). In Thailand, *P. pelagicus* for direct consumption and for use as a raw material in the processing industry are caught in the Andaman Sea and the Gulf of Thailand. In 2008, the production of *P. pelagicus* was 23,600 tons, a decrease of 20.10% compared to production in 2004 and the downward trend is continuing (Department of Fisheries, 2010). Therefore, the culturing of *P. pelagicus* is believed to be a way to increase productivity without placing undue pressure on the wild stock and farmers' job stability for commercial crab culture.

Currently, in Thailand, *P. pelagicus* culture methods of breeding, nursing and rearing to gain higher productivity and survival rates have been developed. The method of rearing *P. pelagicus* broodstock in an earthen pond is also well developed (Oniam *et al*., 2009; 2010). However, studies on the seed production of *P. pelagicus* from broodstock in earthen ponds are limited. Therefore, it remains necessary to study the factors that affect the quality of *P. pelagicus* broodstock cultured in earthen ponds.

Studies on crustacean broodstock nutrition began concretely during the last decade with the growing demand for controlled reproduction in commercial facilities, because nutrition of crustacean broodstock plays a major role in achieving reproductive success and has a considerable influ-

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ence on gonadal maturation, fecundity, egg hatchability and larval viability (Xu *et al*., 1994; Marsden *et al*., 1997; Cavalli *et al*., 1999; Millamena and Quinition, 2000). Thus, the objectives of this experiment were to study fecundity, hatching rate and crab larvae quality of *P. pelagicus* broodstock fed with different feeds. What is learned will be useful for seed production and further development of crab farming, both important for farmers' job stability in the future.

## 2. Materials and Methods

### 2.1 Source of experimental crabs

The experiment was conducted at the hatchery of Klongwan Fisheries Research Station, Prachuap Khiri Khan Province, Thailand. The pond-reared broodstock were the first generation (G1) of wild spawners (G0), which were wild-caught *P. pelagicus* broodstock using crab traps used by small-scale fishermen in the coastal area of Prachuap Bay, Prachuap Khiri Khan Province, Thailand.

In August 2009, female crabs (G1) in pond-reared broodstock conditions (salinity 31-35 ppt, temperature 30.0-34.3°C, dissolved oxygen 4.03-8.96 mg/l, pH 8.18-9.29) had a mean carapace width of 10.76±0.97 cm and body weight of 105.29±32.08 g at about 150 days. All berried females with dark grey eggs (heart-beating stage) were placed in 200-L fiber tanks to allow them to release the eggs for hatching. During this period they were not fed. After hatching, crab larvae were transferred outdoors to 3,000-L concrete tanks for nursing at densities of 100 crabs $L^{-1}$. Newly hatched larvae were initially fed with rotifers (*Branchionus* sp.) and *Chaetoceros* sp. From zoea II stage onwards they were fed with *Artemia* nauplii. Upon larval metamorphosis to the first crab stage they were fed with minced trash fish until the experiment commenced (Oniam *et al*., 2010).

### 2.2 Experimental design and set-up

The experiments were conducted using a completely randomized design (CRD). Crabs with carapace width of 1.5-2.0 cm (about 45 days after hatching) from the nursing concrete tanks were transferred to 400 $m^2$ earthen ponds (20 m length x 20 m width x 1 m depth) at a density of 3 crabs/$m^2$. Crabs broodstock were fed with different feeds. The three treatments were feeding with trash fish, shrimp feed No.4 and mixed feeds (50% trash fish and 50% shrimp feed No. 4) at 5% of body weight per day, twice a day at 0900 hrs and 1600 hrs. The approximate composition of trash fish was 69.53% protein, 9.31% lipid, 5.88% carbohydrate and 14.08% ash (analyzed at Fish Feed Technology Development Center, Faculty of Fisheries, Kasetsart University) and of shrimp feed No. 4 was 37% protein, 4% lipid, 4% fiber and 12% moisture based on nutrient content indicated on the food package. Random samples of 5 berried female crabs with yellow eggs from each treatment were analyzed for tissue composition involving: moisture, crude protein and lipid according to AOAC (2000). Also, samples from 30 berried female crabs with dark grey eggs from each treatment were collected using crab traps after *P. pelagicus* broodstock had been reared in earthen ponds for at least 120 days (Oniam *et al*., 2010), with three replicates per treatment. All berried female samples with dark grey eggs were transferred and placed individually in 200-L fiber tanks for hatching.

To estimate fecundity and hatching rate, the total number of zoea larvae produced was estimated from three 100 mL aliquot water samples taken from the hatching tank. The newly hatched zoea and un-hatched eggs were counted from the sample, and fecundity and hatching rate were calculated using the formula (Arshad *et al*., 2006; Oniam and Taparhudee, 2010)

$$F = HZ + UHE$$

$$HE = \frac{HZ}{F} \times 100$$

where F = fecundity, HE = hatching rate, HZ = total number of the newly hatched zoea, and UHE = total number of un-hatched eggs

Then crab larvae were transferred to 200-L fiber tanks for nursing at densities of 100 crabs/L (5 replicated for each treatments). Survival rates of zoea I (1 day after hatching), megalopa and first crab stages from berried females in earthen ponds fed with different feeds were determined. Survival rate was calculated using the formula :

$$\text{Survival rate} = \frac{\text{number of crab larvae left}}{\text{number of initial crab larvae}} \times 100$$

During the broodstock rearing period, 30% of the water volume was changed once a week. In larval nursing, water exchange was done every three days at about 20-30% to total volume during the zoea I to megalopa stages, and daily water exchange (about 20-30%) was done during the megalopa to first crab stages. Water quality was analyzed twice a week. Salinity was measured by a Refractometer Prima tech, pH by pH meter Cyber Scan pH 11, temperature and dissolved oxygen concentration (DO) by oxygen meter YSI 550A, total ammonia by Koroleff's Indophenol blue method, nitrite by colorimetric method and alkalinity by titration method (APHA, AWWA and WPCF, 2009).

### 2.3 Statistical analysis

At the end of the experiments, the data of fecundity, hatching rate and crab larvae quality were analyzed using analysis of variance (ANOVA) and the difference between means was tested using Duncan's multiple range test (DMRT) at 95% level of confidence using the SPSS program.

## 3. Results and Discussion

In this study, crab broodstock fed with mixed feeds (50% trash fish and 50% shrimp feed) had the highest carapace width (12.07±1.37 cm), carapace length (5.75±0.81 cm) and body weight (138.36±37.51 g) compared to those obtained from crabs broodstock fed with trash fish (10.44±1.51 cm, 4.87±0.73 cm and 90.83±44.54 g, respectively) and shrimp feed (10.25±1.08 cm, 4.89±0.54 cm and 83.23±32.25 g, respectively) (Table 1). The results suggest that the mixed diets support superior growth as was similarly reported by Chaiyawat *et al*. (2008) and Soundarapandian and Dominic Arul Raja (2008).

### 3.1 Fecundity and hatching rate

In this study, minimum fecundity was found in berried female *P. pelagicus* fed with trash fish, shrimp feed and mixed feeds having carapace width of 7.7, 9.1 and 9.1 cm and body weight of 45, 60 and 55 g, respectively. The maximum fecundity were carapace widths of 12.2, 9.5 and 14.3 cm and body weights of 90, 60 and 135 g in berried female crab fed with trash fish, shrimp feed and mixed feeds, respectively. The variation in fecundity is very common in crab and has been reported by many researchers (Erdman and Blake, 1988; Djunaidah *et al*., 2003; Arshad *et al*., 2006; Oniam and Taparhudee, 2010; Wu *et al*., 2010). The hatching rates of female broodstock fed with trash fish, shrimp feed and mixed feeds were 6.81 to 98.16%, 22.69 to 99.03% and 7.94 to 98.04%, respectively. The mean fecundity and hatching rate of *P. pelagicus* broodstock fed with trash fish (569842±243173 eggs and 50.78±25.19%), shrimp feed (464098±188884 eggs and 62.33±26.79%) and mixed feeds (544875±169357 eggs and 50.86±20.76%) were not significant different ($P$>0.05, Table 1).

Fecundity refers to reproductive output, which indicates the number of eggs produced by the animal. The number of eggs produced by females varies with the size of the individual as well as between individuals of a similar size. Generally, larger females produce more eggs than do smaller females (Kangas, 2000). Arshad *et al*. (2006) reported that fecundity of crabs varies from species to species and also varies within the same species due to different factors such as age, size, nourishment, ecological conditions of the water body, etc. Oniam and Taparhudee (2010) reported that the mean fecundity of *P. pelagicus* broodstock was 521,229±195,204 eggs/female and hatching rate was 55.83±24.89%, and that carapace width, carapace length, and body weight were related with fecundity but were not with for hatching rate. The present study yielded similar results. The eggs of *P. pelagicus* were spherical and bright orange when first deposited, but became yellow (cleavage-blastula and gastrula stages), brown (eyespot-pigmentation stages) and dark grey (heart-beating stage) before hatching (Arshad *et al*., 2006; Tangkrock-olan and Champati, 2007). Water temperature and salinity were the main factors affecting hatchability of crab (Yamaguchi, 2001; Hamasaki, 2002; Pinheiro and Hattori, 2003).

### 3.2 Crab larvae quality

Larval development from zoea I to megalopa stages took 10-12 days and megalopa to first crab stages took 5-6 days in each treatment. Similar results for this species were reported by Shinkarenko (1979), Arshad *et al.* (2006) and Oniam *et al*. (2010). Zoea produced by female broodstock fed with mixed feeds had a significantly higher survival rate (89.47±5.03%) compared to those produced by female broodstock fed with trash fish (73.40±14.61%) and shrimp feed (67.33±12.80%) ($P$<0.05). However, mean survival rates of megalopa were 30.09±13.97, 30.24±7.15 and 24.19±9.97% and first crab stages were 1.26±0.88, 0.88±0.38 and 1.20±0.51 % from female broodstock fed with trash fish, shrimp feed and mixed feeds, respectively, and were not significantly different ($P$>0.05) (Table 1). Results showed that *P. pelagicus* broodstock reared in earthen ponds fed with different feeds affected the quality of crab larvae (zoea I stage).

The factors that contribute to low survival of crabs larvae were feed (Soundarapandian *et al*., 2007), cannibalism (Moksnes *et al*., 1998; Marshall *et al*., 2005) and environment such as water quality (Romana and Zeng, 2006, 2007), light intensity and photoperiod (Gardner and Maguire, 1998), etc. Oniam *et al*., (2010) reported that zoea produced by female *P. pelagicus* broodstock younger than 120 days had a significantly lower survival rate compared to those produced by older female broodstock. Wu *et al*. (2010) reported that eggs produced by wild-caught swimming crab *Portunus trituberculatus* broodstock had significantly higher hatchability as well as shorter embryonic development time and crab larvae of zoea I stage had a significantly higher survival rate than that of pond-reared *P. trituberculatus* broodstock. In addition, the causes of crab larvae mortality were moult death syndrome, bacterial disease, parasites and fungi (Morado, 2011; Wang, 2011). Marshall *et al*. (2005) and Maheswarudu *et al*. (2008) reported that the factors that contribute to low survival of megalopa to first crab stages were moult death syndrome (MDS, death associated with moulting) and cannibalism. While MDS may have occurred in the ponds, cannibalism was the main factor affecting mortality.

### 3.3 Egg chemical compositions

The results of analyzing egg chemical compositions showed that the mean moisture, protein and lipid contents of *P. pelagicus* broodstock fed with trash fish were 66.91±2.52, 59.74±1.96 and 20.93±1.67%, fed with shrimp feed 65.78±2.11, 58.84±1.20 and 19.54±2.98% and fed with mixed feeds 67.69±2.36, 60.51±1.80 and 19.48±2.36%, respectively. The moisture, protein and lipid contents of eggs produced by *P. pelagicus* fed with trash fish, shrimp feed and mixed feeds were not significantly different ($P$>0.05) (Table 2).

Table 1. Carapace width, body weight, fecundity, hatching rate and crab larvae quality of *P. pelagicus* broodstock reared in earthen ponds for about 150 days (mean ± SD.).

| Reproductive parameters | Feed of crab broodstock | | |
|---|---|---|---|
| | Trash fish | Shrimp feeds No. 4 | Mixed feeds |
| Female broodstock (*n*=30) | | | |
| Carapace width (cm) | $10.44 \pm 1.51^a$ | $10.25 \pm 1.08^a$ | $12.07 \pm 1.37^b$ |
| Carapace length (cm) | $4.87 \pm 0.73^a$ | $4.89 \pm 0.54^a$ | $5.75 \pm 0.81^b$ |
| Body weight (g) | $90.83 \pm 44.54^a$ | $83.23 \pm 32.25^a$ | $138.36 \pm 37.51^b$ |
| Fecundity (eggs/crab) | $569842 \pm 243173^a$ | $464098 \pm 188884^a$ | $544875 \pm 169357^a$ |
| Hatching rate (%) | $50.78 \pm 25.19^a$ | $62.33 \pm 26.79^a$ | $50.86 \pm 20.76^a$ |
| Crab larvae quality of female broodstock (*n*=5) | | | |
| Survival of zoea I | $73.40 \pm 14.61^a$ | $67.33 \pm 12.80^a$ | $89.47 \pm 5.03^b$ |
| Survival of megalopa | $30.09 \pm 13.97^a$ | $30.24 \pm 7.15^a$ | $24.19 \pm 9.97^a$ |
| Survival of first crab | $1.26 \pm 0.88^a$ | $0.88 \pm 0.38^a$ | $1.20 \pm 0.51^a$ |

Note: Means within the same row with different superscripts are significantly different ($P<0.05$), "*n*" : number of samples.

Table 2. Moisture (% wet weight), protein and lipid (% dry weight) of the eggs (cleavage-blastula stages) of *P. pelagicus* broodstock reared in earthen ponds for about 150 days (mean ± SD.).

| Egg composition | Feed of crab broodstock | | |
|---|---|---|---|
| | Trash fish | Shrimp feeds No. 4 | Mixed feeds |
| Moisture | 66.91 ± 2.52 | 65.78 ± 2.11 | 67.69 ± 2.36 |
| Protein | 59.74 ± 1.96 | 58.84 ± 1.20 | 60.51 ± 1.80 |
| Lipid | 20.93 ± 1.67 | 19.54 ± 2.98 | 19.48 ± 2.36 |

### 3.4 Water quality

The results of water quality in the earthen ponds revealed that salinity ranged from 26 to 30 ppt, water temperature ranged from 28.5 to 32.3°C, DO ranged from 4.23 to 8.49 mg/l, pH ranged from 8.15-9.10 to 8.4, total ammonia ranged from 0.000 to 0.086 mg-N/l, nitrite ranged from 0.000 to 0.021 mg-N/l and alkalinity ranged from 104 to 139 mg/l as $CaCO_3$, and did not affect growth and sexual maturity of *P. pelagicus* (Lignot *et al*., 2000; Romano and Zeng, 2007; Maheswarudu *et a*l., 2008; Oniam *et al*., 2010).

In addition, water quality during the nursing periods (salinity 30 ppt, temperature 27.2-29.5°C, DO 5.04-6.29 mg/l, pH 7.98-8.23, total ammonia 0.000-0.226 mg-N/l, nitrite 0.001-0.721 mg-N/l and alkalinity 114-127 mg/l as $CaCO_3$) did not affect development stages and survival rates of *P. pelagicus* larvae (Arshad *et al*., 2006; Oniam *et al*. 2009).

In most of the studies in crustaceans, growth rate was mainly dependent on the nutritional quality of feeds. Research on nutrient requirements for broodstock maturation relies greatly on formulated diets. Also, for commercial applications, artificial diets are preferred (Djunaidah *et al*., 2003). Crustacean broodstock in captivity are generally fed with chopped fresh food, which has a high nutritional value and is generally regarded as superior to compound diets (e.g. *Penaeus indicus*: Cahu *et al*. 1995; crayfish *Procambarus clarkia*: Laufer *et al*., 1998). However, fresh food decays rapidly and easily deteriorates water quality. Various authors have successfully used dry artificial broodstock diets at a 50% substitution level of the total feeding regime such as for *Penaeus monodon* (Millamena *et al*., 1986), white shrimp *Litopenaeus vananmai* (Wouters *et al*., 2002), and mud crab *Scylla serrata* (Millamena and Quinito, 2000), etc. Marsden *et al*. (1997) reported, however, that better results on reproductive performance were obtained when shrimp broodstock were fed solely (100%) artificial diets containing minced fresh food.

## 4. Conclusion

This study showed that fecundity and hatching rates of *P. pelagicus* broodstock fed with trash fish, shrimp feed and mixed feeds were not significantly different, but zoea I produced by female broodstock fed with mixed feeds had a significantly higher survival rate compared to those produced by female broodstock fed with trash fish and shrimp feed. However, more extensive research has to be done to determine the effects of essential nutrients such as dietary protein level and quality, HUFA levels and amino acids on reproductive performance of *P. pelagicus* broodstock reared in earthen ponds.

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