Reproductive Performance and Larval Quality of First and Second Spawning of Pond-Reared Blue Swimming Crab, *Portunus pelagicus*, Broodstock

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ABSTRACT

The current was conducted to compare the reproductive performance and larval quality of first (1st) and second (2nd) spawning produced by female blue swimming crab, *Portunus pelagicus*. The results showed that there were significant differences in several important reproductive parameters and larval quality, 2nd spawning had lower fecundity (508,657 eggs for 1st spawning and 395,118 eggs for 2nd spawning), a lower total number of zoea larvae (259,985 crabs for 1st spawning and 160,900 crabs for 2nd spawning), a lower hatching rate (54.01% for 1st spawning and 41.41% for 2nd spawning) and a lower survival rates of zoea I (87.87% for 1st spawning and 67.19% for 2nd spawning) than 1st spawning. However, the survival rates of the megalopa (30.94% for 1st spawning and 20.29% for 2nd spawning) and first crab stages (1.57% for 1st spawning and 0.85% for 2nd spawning) were not significantly different. Similar crab larval development of 1st and 2nd spawning from the zoea I to megalopa stages (11.2 d for 1st spawning and 11.4 d for 2nd spawning) and the megalopa to first crab stages (4.8 d for 1st spawning and 5.0 d for 2nd spawning) were not significantly different. This study contends that 2nd spawning of female *P. pelagicus* affected the reproductive performance and survival rates of zoea I.
Keywords: *Portunus pelagicus*, reproduction, first spawning, second spawning

INTRODUCTION

The blue swimming crab (*Portunus pelagicus*), a commercially important species, is distributed throughout the coastal waters of the tropical regions of the western Indian Ocean and the Eastern Pacific while 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 (Department of Fisheries, Thailand 2011). Annual exportation of fresh, chilled, or frozen crabs to

the USA, Japan, Taiwan, and other countries, is a multi-million dollar revenue business for Thailand (Department of Fisheries, Thailand 2011). However, due to overfishing and marine pollution, the natural resource and fisheries production of *P. pelagicus* have shown downward trends in the Andaman Sea and the Gulf of Thailand since 1999; for example, in 2009, the production of *P. pelagicus* was 23,800 t, a decrease of 57.76% or an average of 5.77% per year compared to 1999 (Department of Fisheries, Thailand 2011). Therefore, the culturing of *P. pelagicus* is believed

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to be one way of increasing the productivity without placing undue pressure on the wild stock and on the stability of farmers' jobs.

Currently, in Thailand, *P. pelagicus* culturing methods of breeding, nursing and rearing have been developed to gain higher productivity and survival rates. The method of rearing *P. pelagicus* broodstock in an earthen pond is also well developed (Oniam *et al.*, 2009; 2010), but under rearing conditions, the survival rate was still low and most of the broodstock were small and the pond-reared broodstock could not be maintained over three generations (Oniam and Arkronrat, 2013). In aquaculture, the use of pond-reared spawners has several advantages, including cheaper operational costs, year-round availability, less seasonal variation and the implementation of genetic selection programs. There is increasing interest in the utilization of pond-reared spawners to replace wild-caught broodstock in commercial crab hatcheries (Wu *et al.*, 2010). To produce and utilize domesticated broodstock is an important first step for the seed production and development of crab farming. Many brachyuran crabs can spawn more than once after a single copulation and within a molt period during a reproductive season (Millamena and Quinitio, 2000; Kobayashi, 2001; Dickinson *et al.*, 2006; Nan *et al.*, 2006; Yao *et al.*, 2007). Thus, the objectives of this experiment were to study the reproductive performance and crab larvae quality of first and second spawning female *P. pelagicus* from earthen ponds. The knowledge gained from the research will be useful for the seed production and development of crab farming in Thailand, which are both important for the future job stability of farmers.

MATERIALS AND METHODS

Source of experimental crabs

The experiment was conducted with pond-reared broodstock in the hatchery of the Klongwan Fisheries Research Station, Prachuap Khiri Khan province, Thailand. Broodstock-reared crabs with a carapace width of 1.5–2.0 cm (about 45 d after hatching) from the nursing concrete tanks were transferred to 400 m^2 earthen ponds (20 m length × 20 m width × 1 m depth). The crabs were randomly assigned to two earthen ponds at a density of 3 crabs.m^{-2}. The crab broodstock were fed with mixed feeds (50% trash fish, fresh *Amblygaster* sp., *Selaroides* sp., *Gazza* spp. among others and 50% shrimp feed No. 4) at 5% of body weight per day, twice a day at 0900 and 1600 hours for about 150 d (Oniam *et al.*, 2012). The approximate composition of shrimp feed No. 4 was 37% protein, 4% lipid, 4% fiber and 12% moisture based on the nutrient content indicated on the food package. During the broodstock rearing period, 30% of the water volume was changed once a week.

Experimental design and set-up

The experiment consisted of two treatments nominated as first spawning and second spawning, respectively. Random samples of berried female crabs with dark gray eggs (at least 50 crabs) were collected using crab traps after these crabs had been reared in the earthen ponds for at least 120 d (Oniam *et al.*, 2010). All berried female samples were placed individually in 200 L fiber tanks to allow them to release the eggs for hatching (first spawning). During this period they were not fed. After hatching occurred, the female crabs were reared according to Oniam and Arkronrat (2012). The female crabs were reared individually in plastic baskets in 2,000 L concrete ponds, at 8 baskets per pond. On the bottom of the plastic baskets, sand substrate (around 10 cm thick) was provided for the female crabs to bury in and to facilitate the attachment of eggs to their abdomens during the ovarian re-maturation.

Throughout the experiment, a static water depth of about 80 cm was maintained and 50% of the water was exchanged each morning in each pond. Feces and uneaten feed were removed daily by siphoning. All crabs were fed daily at about 1600 hours with mixed feeds (50% trash fish, fresh

Selaroides sp. and 50% shrimp feed No.4) at 5% of body weight, for 30 d. During the experiment, any spawning and mortality were checked and recorded each morning. When any berried females were found, their date of ovarian re-maturation was recorded before they were transferred to the hatching tank (second spawning).

Reproductive performance

For each female crab, the following data were recorded: carapace width (in centimeters), carapace length (in centimeters) and body weight (in grams). The carapace width was measured between the tips of the epibranchial spines using vernier calipers. The body weight was measured by digital weighing. The reproductive performance, to estimate the fecundity and hatching rate of the first and second spawning of female crab, and the total number of zoea larvae produced were estimated from three 100 mL aliquot water samples taken from the hatching tank. The newly hatched zoea and unhatched eggs were counted from the sample, and the fecundity and hatching rate were calculated using Equations 1 to 5 from Arshad *et al.* (2006) and Oniam and Taparhudee (2010):

Fecundity total number of newly = hatched zoea + total number of un-hatched eggs
(1)

Hatching rate = total number of the newly hatched zoea × 100/fecundity   (2)

The percentage mortality, second spawning rate and berried females successfully hatched were determined and calculated as follows:

Mortality rate = 100 – (number of died females after first spawning

× 100 / total number of females at the beginning of the experiment)   (3)

Second spawning rate = number of berried female found

× 100 / total number of surviving females   (4)

Berried females successfully spawned = number of females spawned

× 100 / total number of berried females
(5)

Crab larvae quality

After hatching, the crab larvae were transferred outdoors to 3,000 L concrete ponds for nursing at a density of 100 crabs.L^{-1} (with five replicates for both treatments). Newly hatched larvae were initially fed with rotifers (*Branchionus* sp.) and *Chaetoceros* sp. Then, from the zoea II stage onward, they were fed with *Artemia* nauplii. In larval nursing, about 30% of the total water volume was exchanged every 3 d during the zoea I to megalopa stages, while during the megalopa to first crab stages there was about 30% daily water exchange.

The larval development and survival are two important indicators of offspring quality (Wu *et al.*, 2010; Oniam *et al.*, 2012). The larval development and survival rates of the zoea I (1 d after hatching), megalopa, and first crab stages from the first and second spawning of female crabs were determined. The larval development was determined using a dissecting microscope at 10× magnification to ensure that the larvae had reached the stage within the time period. The larval stages were differentiated by identification of the telson, eye-stalk, abdomen segmentation, spine and setae according to Arshad *et al.* (2006). The survival rate was calculated using Equation 6:

Survival rate = number of crab larvae left × 100 / number of initial crab larvae   (6)

Water analysis

During the experiment, the water quality was analyzed twice a week. Salinity was measured using a refractometer (Primatech; Jakarta, Indonesia), pH using a portable pH meter (pH 11; CyberScan; Nijkerk, the Netherlands), temperature and dissolved oxygen concentration (DO) using a oxygen meter (550A; YSI Inc.; Yellow Springs, OH, USA), and total ammonia, nitrite and alkalinity using the indophenol blue method, the colorimetric method and the titration

method, respectively (American Public Health Association *et al.*, 2005).

Statistical analysis

At the end of the experiments, the data on reproductive performance and crab larvae quality were analyzed using analysis of variance and the difference between the first spawning and second spawning was tested using a *t*-test at the 95% level of confidence using the SPSS program (Version17; SPSS Inc,; Chicago, IL. USA).

RESULTS

Reproductive performance

The female *P. pelagicus* reared in the ponds under broodstock conditions for the first brood and second brood had a mean carapace width of 10.45 ± 1.21 and 10.59 ± 1.29 cm, a carapace length of 4.95 ± 0.61 and 5.02 ± 0.68 cm and a body weight of 91.12 ± 38.88 and 96.87 ± 40.84 g, respectively. The carapace width, carapace length and body weight of the first and second spawning of *P. pelagicus* broodstock were not significantly different. The mean fecundity or number of eggs per female, the mean total number of zoea larvae and the mean hatching rate of first and second spawning were significantly different, the first spawning had a higher fecundity (508,657 $\pm$ 195,526 eggs), total number of zoea larvae ($259,985 \pm 137,204$ crabs) and hatching rate ($54.01 \pm 24.47\%$) than the second spawning ($395,118 \pm 143,441$ eggs, $160,900 \pm 102,314$ crabs and $41.41 \pm 21.62\%$, respectively) as shown in Table 1.

Under the experimental conditions, the female crabs had achieved re-maturation or developed to the berried of cleavage-blastula stage (eggs yellow) again after a mean time of 10.8 ± 2.1 d after first spawning. In addition, the percentage mortality, second spawning rate and berried females successfully hatched from the second brood of *P. pelagicus* broodstock were 8.93, 80.39 and 75.61%, respectively (Table 1).

Crab larvae quality

The zoea produced by the first spawning of the *P. pelagicus* broodstock had a significantly higher survival rate ($87.87 \pm 6.31\%$) compared to those produced by second spawning ($67.19 \pm 12.77\%$). However, the mean survival rates of megalopa were 30.94 ± 9.51 and $20.29 \pm 8.13\%$, and at the first crab stages were 1.57 ± 0.72 and $0.85 \pm 0.40\%$ from first and second spawning, respectively, and were not significantly different. The mean larval development of the first and second spawning from the zoea I to megalopa stages were 11.2 ± 0.8 and 11.4 ± 0.5 d and from the megalopa to first crab stages were 4.8 ± 0.4 and 5.0 ± 0.7 d, respectively (Table 1).

Water quality

The results from the water quality analysis in the broodstock ponds showed the following levels: salinity ranged from 31 to 32 parts per trillion (ppt), water temperature ranged from 29.0 to 31.1 °C, DO ranged from 4.50 to 5.53 mg.L^{-1}, pH ranged from 8.08 to 8.29, total ammonia ranged from 0.000 to 0.259 milligrams of N.L^{-1}, nitrite ranged from 0.000 to 0.441 milligrams of N.L^{-1}, and alkalinity ranged from 119 to 137 mg.L^{-1} as CaCO$_3$. In addition, the water quality during the nursing periods had the following levels: salinity 31–32 ppt, water temperature 29.1–30.8 °C, DO 4.56–5.61 mg.L^{-1}, pH 8.17–8.64, total ammonia 0.000–0.219 mg.L^{-1}, nitrite 0.000–0.421 mg.L^{-1} and alkalinity 107–132 mg.L^{-1} as CaCO$_3$.

DISCUSSION

The study showed that the fecundity, total number of zoea larvae, hatching rate and survival rates of zoea I (1 d after hatching) from second spawning were significantly lower than those of first spawning for the blue swimming crab, *P. pelagicus*. In addition, the water quality in this study did not affect re-maturation (developed berried stage) and the hatching rate of *P. pelagicus*

Table 1 Reproductive performance and crab larvae quality of first and second spawning of pond-reared blue swimming crab, *P. pelagicus*, broodstock.

Parameter observed	First spawning	Second spawning	*t*-test (*P*-value)
Female broodstock (n = 56)			
Carapace width (cm)	10.45 ± 1.21	10.59 ± 1.29	0.598
Carapace length (cm)	4.95 ± 0.61	5.02 ± 0.68	0.619
Body weight (g)	91.12 ± 38.88	96.87 ± 40.84	0.518
Fecundity (eggs per crab)	508,657 ± 195,526	395,118 ± 143,441	0.005
Total number of zoea larvae (crabs)	259,985 ± 137,204	160,900 ± 102,314	0.0007
Hatching rate (%)	54.01 ± 24.47	41.41 ± 21.62	0.018
Re-maturation time (d)	-	10.8 ± 2.1	-
Mortality rate	-	8.93%	-
Second spawning rate	-	80.39%	-
Berried females successfully spawned	-	75.61%	-
Crab larvae quality	*(n = 5)*	*(n = 5)*	
Survival of crab larvae (%)			
zoea I	87.87 ± 6.31	67.19 ± 12.77	0.011
Megalopa	30.94 ± 9.51	20.29 ± 8.13	0.093
first crab	1.57 ± 0.72	0.85 ± 0.40	0.091
Larval development (d)			
zoea I-megalopa	11.2 ± 0.8	11.4 ± 0.5	0.666
megalopa-first crab	4.8 ± 0.4	5.0 ± 0.7	0.607

Data are represented as mean ± SD; *n* = number of samples.

which was consistent with the results reported by Oniam and Taparhudee (2010) and Oniam and Arkronrat (2012), and did not affect development stages and survival rates of *P. pelagicus* larvae which was consistent with the results reported by Arshad *et al.* (2006) and Oniam *et al.* (2009; 2010). The current results showed that second spawning of female *P. pelagicus* affected the reproductive performance and survival rates of zoea I.

Many brachyuran crabs, including the mud crab, *Scylla serrata* (Millamena and Quinitio, 2000), the Japanese mitten crab, *Eriocheir japonicus* (Kobayashi, 2001), the blue crab, *Callinectes sapidus* (Dickinson *et al.*, 2006), the Chinese mitten crab, *Eriocheir sinensis* (Nan *et al.*, 2006) and the swimming crab, *Portunus trituberculatus* (Yao *et al.*, 2007), can spawn more than once after a single copulation and within an molt period during a reproductive season. It has been reported that for *E. japonicus* and *E. sinensis*, significantly lower fecundity per female was found for the second brood compared to the first brood from the female crabs (Kobayashi, 2001; Nan *et al.*, 2006), which is very similar to what was found in the present study for female *P. pelagicus*. In contrast, the fecundity of some brachyuran crabs did not show significant differences between the two broods, for example, *S. serrata* (Millamena and Quinitio, 2000) and *P. trituberculatus* (Wu *et al.*, 2010).

In addition, fecundity refers to the 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 the fecundity of

crabs varied from species to species and also varied within the same species due to different factors such as age, size, nourishment and ecological conditions of the water body among others. Oniam and Taparhudee (2010) reported that the mean fecundity of *P. pelagicus* broodstock was 521,229 ± 195,204 eggs per female and the hatching rate was 55.83 ± 24.89% with the carapace width, carapace length and body weight being related to fecundity but not related to the hatching rate. Water temperature and salinity have been reported as the main factors affecting the hatchability of crabs. For example, Hamasaki (2002) reported that the egg incubation period of *S. serrata* decreased exponentially from 30 to 10 d with increasing mean temperature in the range 20.3–30.0 °C. Pinheiro and Hattori (2003) reported that the optimum water temperature and salinity for the hatchability of mangrove crab, *Ucides cordatus*, were 27 °C and 15 ppt, respectively. In contrast, for *P. pelagicus*, the optimum water temperature and salinity for hatchability were 28–30 °C and 30–32 ppt, respectively (Arshad *et al*., 2006).

In the current experiment, during the second brood rearing period, re-maturation of female *P. pelagicus* was similar to the studies reported earlier (for example, Oniam and Arkronrat, 2012) with a re-maturation of 7–13 d. In some female brachyuran crabs, the second spawning duration of 20–30 d (e.g. *Scylla serrata*: Millamena and Quinitio, 2000; *Callinectes sapidus*: Dickinson *et al*., 2006; *Portunus trituberculatus*: Wu *et al*., 2010), may be explained by the inherent biological differences among various crab species. For the female *P. trituberculatus*, the larger or older crabs were generally capable of having more spawning within an molt period than smaller or younger crabs. As a result, under captive conditions and depending on the culture conditions and the size of the females, *P. trituberculatus* could spawn two to five times after a single copulation during a spawning season (Yao *et al*., 2007; Wu *et al*., 2010).

The larval development of the first and second spawning from zoea I to megalopa stages and megalopa to first crab stages in the current study were similar to the results for the same species reported by Arshad *et al*. (2006) and Oniam *et al*. (2010). Crab larvae produced by the second brood had different survival rates of zoea I or 1 d after hatching, but not for the survival rates of megalopa and first crab stages, which may have been caused by the broodstock diets. Wu *et al*. (2010) reported that for the newly hatched zoea I larvae of *P. trituberculatus*, lipids were used as the preferred energy source when the larvae were subjected to starvation. Therefore, larvae with a lower total lipid content were expected to be less resistant to starvation, especially the larvae from the second brood because the nutrition of crab broodstock plays a major role in achieving reproductive success and has a considerable influence on larval viability (Millamena and Quinition, 2000; Wu *et al*., 2010). Oniam *et al*. (2012) reported that zoea produced by *P. pelagicus* broodstock fed with mixed feed (50% trash fish and 50% shrimp feed No.4) had a significantly higher survival rate compared to those produced by broodstock fed trash fish or shrimp feed. Larval survival is an important indicator for seed production. In the current study, 0.85% survival from the zoea to first crab stages was recorded for the second spawning of *P. pelagicus*, which while not significantly different from the first spawning (1.57%), was still at an acceptable level (Oniam *et al*., 2011). However, more extensive research has to be done to determine the effects of essential nutrients such as the dietary protein level and quality, HUFA levels and amino acid on the reproductive performance of *P. pelagicus* broodstock.

The other factors that contributed to the low survival of crab larvae were the feed (Baylon, 2009), cannibalism (Marshall *et al*., 2005), water quality (Romano and Zeng, 2006) and light intensity and photoperiod (Andrés *et al*., 2010). Wu *et al*. (2010) reported that the crab larvae at the zoea I stage produced by

wild-caught *P. trituberculatus* broodstock had a significantly higher survival rate than that of pond-reared *P. trituberculatus* broodstock. In contrast, Oniam *et al*. (2009) reported that the seed production of *P. pelagicus* from wild-caught broodstock and pond-reared broodstock were not significantly different. Zoea produced by pond-reared *P. pelagicus* broodstock younger than 120 d had a significantly lower survival rate compared to those produced by older female broodstock (Oniam *et al*., 2010). In addition, the causes of mortality in crab larvae were molt death syndrome (MDS, death associated with moulting), bacterial disease, and parasites and fungi (Morado, 2011; Wang, 2011). Marshall *et al*. (2005) reported that the factors that contribute to low survival during the megalopa to first crab stages were MDS and cannibalism. While MDS may have occurred in the ponds, cannibalism was the main factor affecting mortality.

CONCLUSION

In the current study, the fecundity, total number of zoea larvae, hatching and survival rates of zoea I were recorded for second spawning, and although these were lower than those from the first spawning, they were still at acceptable levels because the seed production (first crab stage) from the second brood did not have a significantly different survival rate to that from the first brood. Therefore, the second brood of female *P. pelagicus* can be used for seed production. Moreover, there is a possibility to enhance reproductive performance and larval quality by selective breeding programs (Gao *et al*., 2008). This is an interesting point for future study.

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