Breeding Performance of Wild and Domesticated Female Broodstock of Blue Swimming Crab, *Portunus pelagicus* (Linnaeus, 1758)

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ABSTRACT

Despite its economic importance, aquaculture technology for the blue swimming crab, *Portunus pelagicus* (Linnaeus, 1758) is still in its infancy. It is generally accepted that using domesticated broodstock is advantageous due to their adaptation to a captive environment. However, empirical data are lacking on breeding performance of domesticated crabs. Thus, the present study was conducted to evaluate breeding performance of domesticated female blue swimming crab broodstock relative to wild-caught females. A single-pair mating was performed with six mating combinations: G1×W, W×G1, G2×W, W×G2, G3×W, and W×G3, where W = wild and G1, G2, G3 = domesticated broodstock in generations 1, 2 and 3, respectively. Breeding performance results showed that domesticated females were superior to the wild females for survival rate during mating (mortality range was 8.3±7.2–8.3±14.4 % for domesticated females vs. 33.3±7.2–45.8±14.4 % for wild females), spawning rate (45.8±26.0–58.3±19.1 % vs. 25.0±12.5–29.2±14.4 %), and hatching rate (49.4±17.1–60.5±24.0 % vs. 19.8±21.0–36.9±13.8 %). Total number of zoea produced varied both among domesticated broodstock groups (G1, G2, G3) and between domesticated and wild groups. No significant differences were observed for maturation time, egg development time, fecundity, or survival rate of zoea. The present study clearly demonstrates the superiority of domesticated female blue swimming crab broodstock over wild-caught females.

Keywords: Blue swimming crab, Mating, *Portunus pelagicus*, Reproductive performance

INTRODUCTION

The blue swimming crab, *Portunus pelagicus* (Linnaeus 1758), is an important commercial marine crustacean species. It is distributed throughout the coastal waters of tropical regions of the western Indian Ocean and the eastern Pacific (Hamid *et al*., 2016). High demand for this species in many Asian countries including Thailand is due to both local consumption and export (FAO, 2013). Unfortunately, due to overexploitation and habitat destruction, the production of this crab, which is mostly from capture fisheries, has shown a downward trend since 2009 (Hamid *et al*., 2016; DOF, 2019). To cope with this problem, the culture of blue swimming crab is one potential and promising solution.

Recently, technologies associated with blue swimming crab aquaculture have been developed or improved, for instance, breeding methods using wild berried females (Tanasomwang *et al*., 2002; Arshad *et al*., 2006), broodstock rearing in ponds (Oniam *et al*., 2010; Oniam and Arkronrat, 2014; 2015), nursing (Andrés *et al*., 2010; Ravi and Manisseri, 2013; Oniam *et al*., 2015; Taher *et al*.,

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2017) and grow-out culture (Maheswarudu *et al*., 2008; Soundarapandian and Raja, 2008; Azra and Ikhwanuddin, 2015; Oniam *et al*., 2016; 2018). Nevertheless, in most of the studies to date, breeding of blue swimming crabs has depended largely on wild broodstock. In particular, studies on crab broodstock production under hatchery conditions are very limited and still underdeveloped (Arkronrat and Oniam, 2017).

Successful aquaculture should rely on domesticated broodstock in which domestication improves reproductive performance (Doyle, 1983; Osure and Phelps, 2006). Despite the lack of previous reports on domestication of portunid crab species, Wu *et al*. (2010) reported similar fecundity and larval quality of wild-caught and hatchery-reared swimming crab (*Portunus trituberculatus*) broodstock, whereas Oniam and Taparhudee (2010) showed that fecundity of wild blue swimming crabs was higher than that of the captive-reared crabs. Domestication of the blue swimming crab has been occurring at Klongwan Fisheries Research Station, Faculty of Fisheries, Kasetsart University (Oniam and Arkronrat, 2013a), whereby blue swimming crabs have been bred in captive conditions for three generations. However, the reproductive performance of the domesticated broodstock has not been studied. Therefore, the present study investigates the breeding performance of domesticated female broodstock as compared to those of wild-caught broodstock. The knowledge gained from the study will be used for crab broodstock production and the development of crab culture.

MATERIALS AND METHODS

Study site and source of crabs used in experiments

The experiments were conducted at Klongwan Fisheries Research Station, Prachuap Khiri Khan Province, Thailand during February 2018 to December 2019. The wild broodstock (W) of blue swimming crabs were caught by local fishermen using collapsible crab traps in the coastal area of Prachuap Bay, Prachuap Khiri Khan Province, Thailand (11º50´ N, 99º49´ E). The domesticated broodstock was established from at least 50 wild males and at least 50 wild females caught from the aforementioned location (Oniam and Arkronrat, 2013a) in 2018. They were naturally bred in an earthen pond as described by Oniam and Arkronrat (2015). The larvae (G1) were reared until reaching sexual maturation, which took about 120-150 days. Then, the G2 crabs were produced using 50 males and 50 females from G1 as broodstock. The rearing process was repeated and the G3 was produced in 2019 using 50 males and 50 females from G2 as broodstock.

Experimental design and set-up

Six different mating combinations of crab broodstocks (female×male) were performed: 1) G1 female×wild male, 2) wild female×G1 male, 3) G2 female×wild male, 4) wild female×G2 male, 5) G3 female×wild male, and 6) wild female×G3 male. Each mating combination was replicated with 24 pairs of crabs. In this paper, mating pair types are referred to as G1×W, W×G1, G2×W, W×G2, G3×W and W×G3, respectively.

Broodstock preparation

The sexually mature crabs were transferred to the hatchery and individually reared in plastic baskets (20×26×10 cm) placed in 1.2×6.0×1.0 m concrete ponds, with 24 pairs per pond. Only male crabs at the inter-molt stage and female crabs at the pre-molt stage were selected when the redliner had formed on the fifth pereiopod (Arkronrat and Oniam, 2017). The redliner is the final stage before molting, and crabs in this stage usually molt within 3-7 days (Marshall *et al*., 2005). Crabs were fed with trash fish (mainly *Amblygaster* sp., *Selaroides* sp. and *Gazza* spp.) and artificial shrimp feed (37% protein, 4% lipid, 4% fiber and 12% moisture) at 5 % of body weight per day. During the crab fattening period, molting female crabs were collected each morning. To maintain good water quality, approximately half of the water was exchanged once a week.

Mating method of crab broodstocks

Newly molted female crabs (molted within 12 h) were transferred to a 10-L plastic mating tank.

Then, a selected male crab with a similar size to the female crab was released into the tank, with one pair per tank. Normally, mating behavior occurred within 10-20 min, and the copulation process of the crab lasted about 3-4 h (Arkronrat and Oniam, 2017).

Management of female crabs after mating

After mating had occurred (post-copulation), the female crabs were transferred and reared individually in glass aquaria (60×30 cm with 38 cm water depth). Sand substrate (around 5 cm thick) was provided on the bottom of the aquaria for the female crabs to bury in and to facilitate the attachment of eggs to their abdomens during ovarian maturation. All crabs were fed daily at 4:00 p.m. with shrimp feed No. 4S at about 5 % of body weight, for 30 days. Spawning and/or mortality were checked and recorded each morning. When berried females with yellowish orange eggs (cleavage-blastula stages) were found, the day of ovarian maturation was recorded until the egg color changed to dark grey (heart-beating stage). Then, the female was transferred to the hatching tank for spawning.

Crab breeding and nursing methods

Female crabs with dark grey eggs were placed in 200-L fiberglass tanks to allow them to release eggs. They were not fed during this period. The newly hatched crab larvae were transferred to 3,000-L concrete nursery tanks at a density of 100 crabs·L^{-1}. They were initially fed with rotifers (*Brachionus* sp.) and diatoms (*Chaetoceros* sp.). From the zoea II stage onward, they were fed with *Artemia nauplii* until the larvae had metamorphosed to the first crab stage. They were then fed with shrimp feed No. 1 (pellet size about 0.40-0.42 mm, 38 % protein). The young crabs with a carapace width of 1.5-2.0 cm (about 40-45 days after hatching) were transferred from the concrete nursing tanks to broodstock rearing ponds.

Data collection

The crabs used in the experiment were individually measured for carapace width (CW, in cm), carapace length (CL, in cm), and body weight (BW, in g). For female crabs, the percentage mortality after mating and the spawning rate (percentage of berried females successfully hatched) were calculated.

After the eggs had hatched, the total number of newly hatched zoea and total number of unhatched eggs were estimated from three 100-mL aliquot water samples taken from the hatching tank (Oniam *et al*., 2012). The fecundity, hatching rate and total number of zoea I were calculated as follows:

Fecundity = number of newly hatched zoea + total number of unhatched eggs

Hatching rate = number of newly hatched zoea × 100 / fecundity

Survival rate of zoea I = number of crab larvae at 1 day after hatching × 100 / number of newly hatched zoea.

Water quality management

During the experiments, approximately one-half of the water in the egg development tank was exchanged once a week. Water quality parameters were monitored twice a week. Salinity was determined using a refractometer (Prima tech), and the pH of the water was measured using a portable pH meter (Cyber Scan pH 11). The dissolved oxygen concentration (DO) and temperature of the water were measured using an oxygen probe (YSI 550A), and the total ammonia, nitrite and alkalinity of the water were determined using the indophenol blue method, the colorimetric method, and the titration method, respectively (APHA, AWWA and WEF, 2017).

Statistical analysis

At the end of the experiments, descriptive statistics of all measurements were calculated. The data on breeding performance (fecundity, hatching rate, number of zoea produced and survival rate of crab larvae) were analyzed using one-way ANOVA and the difference between means was tested using Duncan's multiple range test at the 95 % level of confidence by IBM SPSS Statistics for Windows software (version 21.0; IBM Corp., Armonk, NY. USA).

RESULTS

Water quality parameters measured during the experiment were within suitable ranges: water salinity was 30.5–32.3 psu, DO was 4.61–5.58 $mg \cdot L^{-1}$, water temperature was 27.8-30.1 °C, pH was 8.02-8.37, total ammonia was 0.05-0.23 $mg\text{-}N \cdot L^{-1}$, nitrite was 0.01-0.18 $mg\text{-}N \cdot L^{-1}$ and total alkalinity was 114.0-131.5 $mg \cdot L^{-1}$ $CaCO_3$ (Tanasomwang *et al.*, 2002; Romano and Zeng, 2007; Soundarapandian and Raja, 2008; Oniam and Arkronrat, 2013b; 2014). The water conditions in the egg development tanks did not significantly differ among the treatments (p>0.05). These parameters appeared not to affect the breeding and rearing of this crab species.

CW, CL and BW of the crab broodstock did not significantly differ (p>0.05) among mating combinations (Table 1). The mortality rates of the domesticated female crabs (G1-G3) were not significantly different from each other (8.3±7.2, 8.3±14.4 and 8.3±7.2 % for G1, G2 and G3, respectively), but they were lower than for the wild female crabs (33.3±7.2-45.8±14.4 %; p = 0.012). The domesticated female crabs showed higher spawning success (58.3±19.1, 45.8±26.0 and 50.0±12.5 % for G1, G2 and G3, respectively) than the wild female crabs (25.0±12.5–29.2±14.4 %; p = 0.001) (Table 2).

The mean times for crab maturation and the development of eggs from the cleavage-blastula to heart-beating stages were not significantly different among mating types (p = 0.962 and p = 0.593, respectively) (Table 2). After mating, the female crabs in each mating type reached a berry stage when embryos were in cleavage-blastula stages (yellow eggs) after 17.3±1.2-18.2±2.1 days, and the embryos developed to the heart-beating stage (dark grey eggs) after 8.8±0.8-10.0±1.3 days.

The fecundity of wild and domesticated female crabs in our experiments was not significantly different (p = 0.465) (Table 2). However, the mean hatching rates of the wild female crabs (mating types W×G1, W×G2 and W×G3) were significantly lower than those of the domesticated females (G1×W, G2×W and G3×W; p = 0.011). The effect of domestication on numbers of zoea/female is unclear from our experimental results. Three of the mating combinations (G1×W, G2×W and W×G2) had significantly greater zoea/female than the combination of W×G3, while other pairs of mating types did not differ. The low mean found for the

Table 1. Carapace width, length and body weight (mean±SD) of female and male broodstock of blue swimming crab, *Portunus pelagicus*, used in six mating combinations.

Traits	Mating combination (female×male)					
	G1×W	W×G1	G2×W	W×G2	W×G3	W×G3
Carapace width (cm)						
Female	10.3±0.7[a]	10.2±0.7[a]	9.9±0.6[a]	10.0±0.9[a]	10.3±0.6[a]	11.2±0.7[a]
Male	10.5±0.8[a]	10.4±0.7[a]	9.7±0.6[a]	10.4±1.0[a]	10.5±0.6[a]	10.9±0.6[a]
Carapace length (cm)						
Female	5.0±0.9[a]	4.9±0.3[a]	4.7±0.3[a]	4.7±0.4[a]	4.9±0.3[a]	5.1±0.4[a]
Male	5.1±0.4[a]	5.0±0.4[a]	4.6±0.2[a]	5.0±0.5[a]	5.0±0.2[a]	4.9±0.3[a]
Body weight (g)						
Female	91.6±9.4[a]	97.7±8.6[a]	94.1±9.4[a]	98.3±10.9[a]	92.0±7.7[a]	95.2±9.2[a]
Male	93.8±10.8[a]	99.6±9.2[a]	90.8±9.1[a]	103.5±10.0[a]	99.7±7.7[a]	90.8±9.5[a]

Note: Means within a row with the same superscripts are not significantly different (p>0.05); W = wild broodstock; G1, G2 and G3 = domesticated broodstock in generations 1, 2 and 3, respectively.

Table 2. Breeding performance parameters (mean±SD) of female broodstock of blue swimming crab, *Portunus pelagicus*, used in six mating combinations.

Traits	Mating combination (female×male)					
	G1×W	W×G1	G2×W	W×G2	W×G3	W×G3
Mortality rate (%)	8.3±7.2^{b}	33.3±7.2^{a}	8.3±14.4^{b}	45.8±14.4^{a}	8.3±7.2^{b}	37.5±0.0^{a}
Spawning rate (%)	58.3±19.1^{a}	25.0±12.5^{b}	45.8±26.0^{a}	29.2±14.4^{ab}	50.0±12.5^{a}	25.0±12.5^{b}
Maturation time (days)	17.8±1.5^{a}	18.0±1.4^{a}	17.3±1.2^{a}	17.8±1.7^{a}	18.0±1.4^{a}	18.2±2.1^{a}
Egg development time (days)	9.2±1.2^{a}	9.5±1.0^{a}	8.8±0.8^{a}	10.0±1.3^{a}	9.3±1.2^{a}	9.3±1.0^{a}
Fecundity (eggs per crab)	243,192±75,765^{a}	308,420±95,235^{a}	293,883±91,753^{a}	286,023±88,663^{a}	226,188±42,012^{a}	258,853±75,145^{a}
Hatching rate (%)	57.8±24.1^{a}	36.6±16.0^{b}	60.5±24.0^{a}	36.9±13.8^{b}	49.4±17.1^{a}	19.8±21.0^{b}
Total number of zoea produced (crabs)	133,500±54,158^{a}	102,750±22,921^{ab}	176,250±88,821^{a}	114,500±84,952^{a}	110,500±37,979^{ab}	39,750±21,187^{b}
Survival rate of zoea I (%)	83.1±7.1^{a}	80.8±7.5^{a}	84.1±6.0^{a}	81.1±6.2^{a}	79.0±5.7^{a}	80.0±7.4^{a}

Note: Means within a row with different superscripts are significantly different ($p<0.05$); W = wild broodstock; G1, G2 and G3 = domesticated broodstock in generations 1, 2 and 3, respectively.

W×G3 combination may suggest that the sperm quality or quantity of the males (G3) in this cross may have been lower than for the other domesticated males (G1 and G2). No statistically significant differences were found in survival rates of the crab larvae (zoea I) among the six mating types (p = 0.770) (Table 2).

DISCUSSION

Our experiments showed that the overall reproductive performance of the domesticated female blue swimming crab was similar to that of the wild females except for spawning and hatching rates, for which the domesticated females were superior to the wild ones. In general, fecundity of blue swimming crab depends on age and size of the females (Xiao and Kumar, 2004; Hamid *et al*, 2016). According to the high correlation between age and size (Arshad *et al*., 2006), it was surmised that the wild and domesticated females used in the present study were of similar age. The present results are in contrast with previous reports by Wu *et al*. (2010), who reported no difference in fecundity of wild swimming crabs and hatchery-reared broodstock (broodstock originated from offspring of wild crabs). We believe that the contradictory results are due to different nutritional values of the feed; the aforementioned study used trash fish as feed, while we supplemented trash fish with artificial shrimp feed in the present study. The artificial feed may have provided additional nutrients, for example, fatty acids (Djunaidah *et al*., 2003), and thus improved fecundity of the domesticated female broodstock, whereas the wild broodstock may have acquired those nutrients from natural foods in the wild. The benefit of adding artificial shrimp feed to trash fish in blue swimming crab broodstock diet was demonstrated by Oniam *et al*. (2012), whereby the fecundity was enhanced about 17.8 % compared to the group solely fed with trash fish. It should be noted that although Oniam and Taparhudee (2010) reported higher fecundity (number of eggs·g^{-1} body weight) of wild broodstock than the hatchery-reared broodstock of blue swimming crab, the wild females used in their study were larger than the hatchery-reared females. Thus, such difference may be because of size-dependent fecundity.

The relative performance of wild and domesticated females in terms of maturation time, egg development period, hatching rate and survival rate of crab larvae has not been reported before. Therefore, the present study provides the first evidence that domesticated blue swimming crabs can perform as well as the wild female crabs in these traits, and even outperform the wild females in hatching rate. In general, domestication improves reproductive performance of the domesticated stocks, as has been shown in other crustaceans, e.g., the Pacific white shrimp, *Litopenaeus vannamei* (Suresh, 2015), the black tiger shrimp, *Penaeus monodon* (Maheswarudu *et al*., 2016), and the giant freshwater prawn, *Macrobrachium rosenbergii* (Khasani *et al*., 2018). This is in line with the hatching rates reported in the present study. Nevertheless, the difference observed in the other traits might be a result of a short domestication period (3 generations) of the crabs in the present study as compared to more than five generations of broodstock in the aforementioned reports (Pullin *et al*., 1998).

Reproductive performance of the blue swimming crab in the present study was not different over successive generations of domestication, but it would be expected to improve providing that broodstock management is appropriate, e.g., using of large number of broodstock, single pair mating and equal family size (Falconer and Mackay, 1996). Using broodstock from crabs reared in earthen ponds for mating, especially females, will likely result in positive effects on their reproductive performance compared to using females from the wild. However, for year-round hatchery operation, the male crab broodstock is also important. Normally, about 50 % of male *Portunus pelagicus* crabs attain sexual maturity when they reach a size of 10-11 cm in carapace width (Soundarapandian *et al*., 2013), which is very similar to the males used in this study (9.7-10.9 cm).

The present results imply that the domesticated stock used in our trials had been properly managed. However, good broodstock management practices should be continually used. In particular, a large number of broodstock should be used to reduce the risk of losing genetic variation and to avoid inbreeding (Falconer and Mackay, 1996). A domesticated stock is beneficial in reducing operational costs, enabling year-round availability with less seasonal variation (Wu *et al*., 2010). Furthermore, this stock is useful for a genetic selection program which will facilitate commercial culture of this species. This is an interesting issue for future study, which should aim for the development of genetic improvement strategies.

CONCLUSION

This study provides the first evidence of the superiority of domesticated female blue swimming crab, *Portunus pelagicus* over wild females. Hatching rates of the domesticated G1-G3 generation female crabs (in the crosses G1×W, G2×W and G3×W) were higher than those of the wild female crabs (in W×G1, W×G2 and W×G3). The number of zoea produced from the wild female crabs (in W×G3) was lower than from the G1-G3 generation female crabs. In addition, the mortality rates of the G1-G3 female crabs after mating were lower than those of wild female crabs.

These findings are an important first step in attaining a sustainable seed supply of blue swimming crab. However, to have more complete information for broodstock management, further research is needed on the reproductive performance of the males. We hope that in the near future, this will lead to the establishment of a selective breeding program that produces the first genetically improved strain of blue swimming crab, possessing desired traits such as fast growth and high survival rates.

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