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Growth Rate and Sexual Performance of Domesticated Blue Swimming Crab, *Portunus pelagicus* (Linnaeus, 1758) in Earthen Ponds

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Abstract

It is generally accepted that using domesticated aquatic animal is advantageous due to their adaptation to a captive environment. Despite previous reports on the domestication of the blue swimming crab, *Portunus pelagicus* (Linnaeus 1758), empirical data are lacking on the growth and sexual performance of domesticated individuals of this crab species. Thus, the growth and sexual performance were evaluated of domesticated *P. pelagicus* from the 1st to the 3rd generations. Domesticated crabs in the 1st (G1), 2nd (G2) and 3rd (G3) generations were reared for 150 days in 400 m^2 earthen ponds at a density of 2 crabs/m^2. Each generation had 3 replicates. The performance results showed that the mean final CW (11.4 cm) of G1 was higher than in G3 (9.9 cm), and G1 had a higher mean final BW (123.4 g) and SGR (4.8 % day^{-1}) than in G2 (108.2 g and 4.7 % day^{-1}, respectively) and G3 (102.9 g and 4.7 % day^{-1}, respectively). The mean SR values in G1 (20.2 %), G2 (18.8 %) and G3 (18.3 %) were not significantly different. No significant differences were observed in mean FCR and productivity in G1 (5.8 and 94.1 kg/pond, respectively), G2 (6.6 and 82.9 kg/pond, respectively) and G3 (6.7 and 82.0 kg/pond, respectively). Sexual dimorphism in G1, G2 and G3 was observed at 60 days of rearing, with the sex ratio being 1:1 in each generation and 5.6 and 6.7 % of female crabs in G2 and G3, respectively, being berried in this period. In contrast, the berried females in G1 (17.6 % of females) were 1st found on day 90 of the rearing period. At day 150, the percentage composition ranges for yellow, brown and dark gray egg colors of the reared berried female crabs were 20.0 - 25.0, 40.0 - 50.0 and 25.0 - 40.0 %, respectively. These finding should assist in the future establishment of a selective breeding program that produces the 1st genetically improved strain of *P. pelagicus* possessing desired traits such as fast growth and high survival rates.

Keywords: *Portunus pelagicus*, Domestication, Growth, Sexual maturity

Introduction

The blue swimming crab, *Portunus pelagicus* (Linnaeus 1758), is an important commercial marine crustacean species. It is distributed throughout the coastal waters of the tropical regions of the western Indian Ocean and the eastern Pacific Ocean [1]. High demand for this species in many Asian countries including Thailand is due to both local consumption and export. 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 [2]. 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 [1,3]. To cope with this problem, the culture of *P. pelagicus* is one potential and promising solution. Many countries have been actively involved in *P. pelagicus* culture and associated research, including the Philippines, Indonesia, India, Australia, Malaysia and Thailand [4-8]. Despite its economic importance, currently, the culture techniques for *P. pelagicus* have not yet been fully established to expand the farms and increase productivity to a commercial scale [6,7].

Studies on the culture of *P. pelagicus* have found some of the main causes of low productivity include cannibalism [9,10], food and feeding [11,12] and the quality of the bottom soil of the pond [13]. Nevertheless, in most of the studies to date, *P. pelagicus* rearing has depended largely on wild broodstock. It is generally accepted that using domesticated aquatic animals is advantageous due to their adaptation to a captive environment, thus successful aquaculture should rely on domesticated broodstock as domestication improves reproductive and growth performances [14]. The main process in domestication is repeated selection to produce the best quality generation under the culture conditions [15], as has been applied successfully with other crustaceans such as the Pacific white shrimp, *Litopenaeus vannamei* [16], the black tiger shrimp, *Penaeus monodon* [17], and the giant freshwater prawn, *Macrobrachium rosenbergii* [18].

Despite the lack of previous reports on domestication of portunid crab species, domestication of *P. pelagicus* has been occurring at the Klongwan Fisheries Research Station, Faculty of Fisheries, Kasetsart University [19,20]. Oniam *et al.* [21] reported the superiority of the domesticated female crab, *P. pelagicus* over the wild females. Hatching rates and the number of zoea produced by the domesticated female crabs were higher than those of wild female crabs. Crabs have now been bred in captive conditions for 3 generations. However, empirical data are lacking on the growth rate and sexual performance of the domesticated crabs that have been reared. Therefore, the present study focused on the impact of domestication on the growth and sexual maturity of the crab. The knowledge gained from the study will be used to improve domesticated *P. pelagicus* production and the development of its culture.

Materials and methods

Study site and source of crabs used in experiments

The experiments were conducted at the Klongwan Fisheries Research Station, Prachuap Khiri Khan province, Thailand during February 2018 to December 2019. The wild broodstock (W) of blue swimming crabs (*P. pelagicus*) were caught by local fishermen using collapsible crab traps in the coastal area of Prachuap Bay, Prachuap Khiri Khan province, Thailand (11°50´ N, 99°49´ E). The domesticated broodstock was established from at least 50 wild males and at least 50 wild females caught from the aforementioned location [19] in 2018. They were naturally bred in an earthen pond as described by Oniam and Arkronrat [20]. 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 stock was produced in 2019 using 50 males and 50 females from G2 as broodstock.

Female crabs with dark gray eggs of W, G1 and G2 were placed in separate 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/m^2. 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 1st crab stage. Then, they were fed with shrimp feed No. 1 (pellet size approximately 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 crab rearing ponds.

Experimental design and set-up

The experiment was designed using 3 treatments to investigate the impact of domestication on the growth and sexual maturity of *P. pelagicus* culture for 1st (G1), 2nd (G2) and 3rd (G3) generation crabs. Experimental crabs in G1, G2 and G3 from the concrete nursing tanks were transferred to separate 400 m^2 earthen ponds with aeration, at a density of 2 crabs/m^2. Each treatment had 3 replicates, for 150 days.

The crabs were fed artificial shrimp feed according to Oniam *et al*. [22]. During the first 30 days of the 120-day experimental culture period, the crabs were fed shrimp feed No. 2 (STARTEQC™, pellet size 0.8 - 1 mm, 38 % protein) at 30 % of body weight per day. At 31 - 60 days of culture, the crabs were fed shrimp feed No. 4S (STARTEQC™, pellet size 3.5 mm, 38 % protein) at 5 % of body weight per day; then, at 61 - 150 days of culture, crabs were fed with shrimp feed No. 4S at 3 % of body weight per day. Throughout the experiment, crabs were fed twice a day at 0900 h and 1700 h.

Data collection

The crabs used in the experiment were individually measured for carapace width (CW, in cm), and body weight (BW, in g). The CW was measured between the tips of the epibranchial spines using vernier calipers. The BW was measured using a set of digital weighing scales. Random samples of 50 crabs from each earthen pond collected using a crab trap were evaluated for their growth and sexual maturity every 30 days according to Oniam *et al*. [23].

During the experiments, approximately one-half of the water in each earthen pond 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 [24]. In addition, the bottom soil quality was analyzed once a month by collecting bottom soil samples from 2 places in each earthen pond using a 5 cm diameter, clear plastic, core liner tube (Wildlife Supply Company, Buffalo, NY, USA). The upper 5 cm segment of each core was removed as described by Munsiri *et al*. [25], and 2 core segments from each pond were combined to provide a composite sample for analysis. Tests were undertaken for organic matter concentration using the ignition loss method [26]. Soil pH was measured using the method described by Thunjai *et al*. [27] and the total ammonia concentration was determined using the method described by Chuan and Sugahara [28].

At the end of the rearing period, the surviving crabs were counted, weighed and measured to assess final CW and BW. The specific growth rate (SGR), survival rate (SR), and feed conversion ratio (FCR) were calculated [29,30], using equations 1 - 3, respectively:

$$\text{SGR} (\% \text{ day}^{-1}) = 100\times[\ln (\text{final body weight}) - \ln (\text{initial body weight})] / \text{culture period} \quad (1)$$

$$\text{SR} (\%) = 100\times(\text{final crab number}) / (\text{initial crab number}) \quad (2)$$

$$\text{FCR} = \text{total feed given} / \text{crab weight gain} \quad (3)$$

Data analysis

At the end of the experiments, descriptive statistics of all measurements were calculated. The data on growth (final CW and BW), SGR, SR and FCR and on the water and bottom soil qualities of all treatments were analyzed using one-way ANOVA and differences between means was tested using Duncan's multiple range test at the 95 % level of confidence. All data were analyzed using the IBM SPSS Statistics for Windows software (https://www.ibm.com/support/pages/downloading-ibm-spss-statistics-21). In addition, simple descriptive statistics (mean and percentage) were used to estimate the production potential and sexual maturity.

Results and discussion

Domesticated crab growth performances

The growth performance of the domesticated *P. pelagicus* was determined at 150 days. The final CW, BW and SGR of the crabs in G1 were significantly different from the other generations ($P < 0.05$). They had a higher mean final CW (11.4 cm) than in G3 (9.9 cm) and a higher final BW (123.4 g) and SGR (4.8 % day^{-1}) than in G2 (108.2 g and 4.7 % day^{-1}, respectively) and G3 (102.9 g and 4.7 % day^{-1}, respectively). The mean SR values in G1, G2 and G3 were 20.2, 18.8 and 18.3 %, respectively. The SR values were not significantly different ($P > 0.05$) (**Table 1**).

The mean FCR values of the crabs reared in the 400 m^2 earthen ponds were in the range 5.8 - 6.7 and the productivity was in the range 82.0 - 94.1 kg/pond. There were no significant differences among the FCR and productivity values among the generations ($P > 0.05$) (**Table 1**).

Table 1 Performance of domesticated blue swimming crab (*P. pelagicus*) reared in earthen ponds for 150 days (mean ± SD).

Treatments	Final CW (cm)	Final BW (g)	SGR (% day^{-1})	SR (%)	FCR	Productivity (kg/pond)
G1	11.4 ± 0.3^{a}	123.4 ± 5.0^{a}	4.8 ± 0.1^{a}	20.2 ± 2.8^{a}	5.8 ± 0.7^{a}	94.1 ± 11.4^{a}
G2	10.2 ± 0.8^{ab}	108.2 ± 8.7^{b}	4.7 ± 0.1^{b}	18.8 ± 1.3^{a}	6.6 ± 0.7^{a}	82.9 ± 9.3^{a}
G3	9.9 ± 0.5^{b}	102.9 ± 1.3^{b}	4.7 ± 0.0^{b}	18.3 ± 2.8^{a}	6.7 ± 1.0^{a}	82.0 ± 12.2^{a}

Note: Means within a column with different lowercase superscripts are significantly different ($P < 0.05$); G1, G2 and G3 = 1st, 2nd and 3rd generations, respectively.

Sexual maturity

The percentage composition of non-sexual and sexual maturity of the domesticated *P. pelagicus* reared in earthen ponds are shown in **Figure 1**. Sexual dimorphism of the domesticated crabs was observed at 60 days into the rearing period (21.7 ± 1.7, 27.8 ± 2.1 and 26.7 ± 3.6 % of all female crabs in G1, G2 and G3, respectively) with a sex ratio of 1:1 in all generations.

The berried female crabs were first found on day 90 of the rearing period in G1 (17.6 ± 3.3 % of all female crabs), whereas in G2 and G3 they were first found on day 60 of the rearing period (5.6 ± 1.5 and 6.7 ± 2.2 % of all female crabs, respectively). At day 150, the percentages of yellow, brown and dark gray egg colors of the domesticated crabs in G1 were 25.0 ± 5.0, 50.0 ± 0.0 and 25.0 ± 0.5 %, respectively, of all female crabs, in G2 were 20.0 ± 10.0, 40.0 ± 10.0 and 40.0 ± 0.0 %, respectively, of all female crabs and in G3 were 20.0 ± 17.3, 40.0 ± 10.0 and 40.0 ± 20.0 %, respectively, of all female crabs (**Figure 2**).

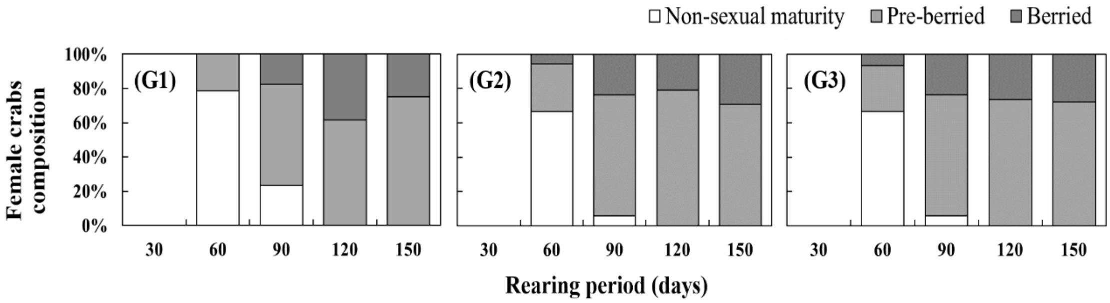

Figure 1 Percentage composition of non-sexual and sexual maturity (pre-berried and berried) of domesticated blue swimming crab (*P. pelagicus*) in 1st (G1), 2nd (G2) and 3rd (G3) generations in earthen ponds.

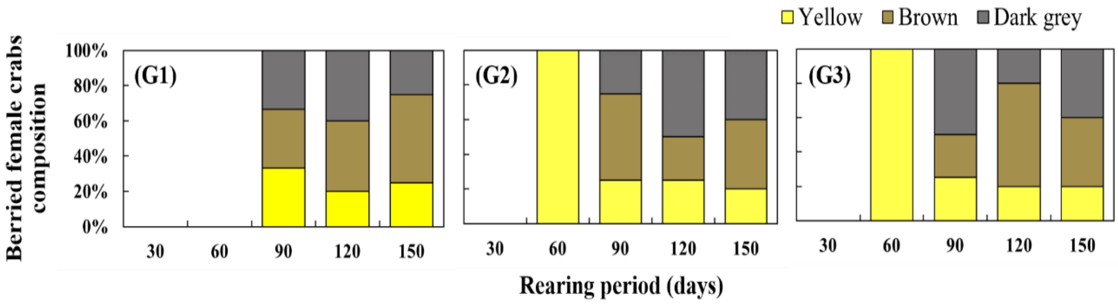

Figure 2 Percentage composition of berried female domesticated blue swimming crabs (*P. pelagicus*) in 1st (G1), 2nd (G2) and 3rd (G3) generations in earthen ponds.

Rearing ponds conditions

The mean values for water quality are shown in **Table 2**. The water conditions in the rearing ponds of the domesticated crab did not significantly differ among the treatments ($P > 0.05$). The water quality parameters measured during the experiment were within suitable ranges, specifically for water salinity (32.1 - 32.8 ppt), water temperature (29.1 - 29.3 °C), DO (5.2 - 5.3 mg/L), pH (8.1 - 8.1), total ammonia (0.2 - 0.3 mg-N/L), nitrite (0.0 mg-N/L) and total alkalinity (109.9 - 112.0 mg/L as $CaCO_3$). These parameters have been reported to not affect the rearing of this crab species [5,6,11,12,31].

The mean soil pH, organic matter, ammonia, and sulfide concentrations in the bottom soil of the earthen ponds sued in the experiment are shown in **Table 3**. The crab pond bottom soil in each generation had the following ranges for soil pH 6.4 - 6.8, organic matter 3.2 - 7.2 %, ammonia 3.4 - 5.5 mg/kg and sulfide 0.09 - 0.13 mg/g by dry weight. These parameters were not significantly different among the treatments ($P > 0.05$) and have been reported to not affect the rearing of this crab species [12,13,30].

Table 2 Water quality parameters during experiment involving domesticated blue swimming crabs (*P. pelagicus*) reared in earthen ponds, for 150 days (mean ± SD).

Parameter	Treatment			*P* - value
	G1	G2	G3	
Salinity (ppt)	32.2 ± 1.0	32.8 ± 1.1	32.1 ± 0.7	0.247
Temperature (°C)	29.1 ± 0.9	29.3 ± 0.7	29.2 ± 0.7	0.865
Dissolved oxygen (mg/L)	5.3 ± 0.5	5.2 ± 0.7	5.2 ± 0.4	0.927
pH	8.1 ± 0.6	8.1 ± 0.5	8.0 ± 0.7	0.178
Total ammonia (mg-N/L)	0.2 ± 0.3	0.3 ± 0.3	0.2 ± 0.2	0.164
Nitrite (mg-N/L)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.155
Alkalinity (mg/L as $CaCO_3$)	112.0 ± 9.2	109.9 ± 7.6	118.4 ± 8.2	0.746

G1, G2 and G3 = domesticated crabs in 1st, 2nd and 3rd generations, respectively.

Table 3 Bottom soil quality parameters during experiment involving domesticated blue swimming crabs (*P. pelagicus*) reared in earthen ponds for 150 days (mean ± SD).

Parameter	Treatments			*P* - value
	G1	G2	G3	
Soil pH	6.4 ± 0.2	6.8 ± 0.4	6.6 ± 0.2	0.138
Organic matter (%)	5.2 ± 1.0	3.2 ± 1.9	7.2 ± 6.2	0.096
Ammonia (mg/kg)	5.5 ± 1.0	4.6 ± 7.8	3.4 ± 1.1	0.091
Sulfide (mg/g by dry weight)	0.13 ± 0.09	0.09 ± 0.07	0.12 ± 0.09	0.164

G1, G2 and G3 = domesticated crabs in 1st, 2nd and 3rd generations, respectively.

Studies on the culture of the blue swimming crab, *P. pelagicus* have reported that the revenue from market crab production (50 - 70 g/crab) in grow-out ponds did not cover the cost because of low growth and survival rates [32]. These problems have been major bottlenecks to the development of commercial aquaculture for this species. Domestication involves the successful acclimatization of a species to rearing conditions that improve the growth and survival of the individuals [14,15] and is one possible solution to overcome these problems with *P. pelagicus* aquaculture.

Our experiments showed that the rearing conditions did not affect the growth and survival rate of domesticated *P. pelagicus* in each generation, but there were significant differences in mean growth between G1 (11.4 cm CW, 123.4 g BW and 4.8 % day^{-1}) and G3 (9.9 cm CW, 102.9 g BW and 4.7 % day^{-1}) indicating that domestication of *P. Pelagicus* had some negative effects by G3. Although the growth through to G3 was slow, the SGR of domesticated crab in each generation of this study showed good prospects for growth compared to culturing of non-domesticated crabs of this species in other studies where the SGR of weights were in the range 1.3 - 3.9 % day^{-1} [6,11,23,32]. In general, the factors that contribute to lower productivity of commercial *P. pelagicus* culture are cannibalism [7,9,10] and the rearing conditions [5,6,11,12]. In addition, Oniam [33] reported that mortality in crab rearing was affected more by the quality of the rearing conditions than cannibalism, especially after 90 days of culture. In the present study, there were no significant differences in the values for SR, FCR and productivity among G1 - G3 of the domesticated *P. pelagicus*. The rearing conditions are an important factor in *P. pelagicus* aquaculture, with the benefit of the higher growth and survival being a shortening of the culture period. Therefore, the use of domesticated stock of *P. pelagicus* in the 1st - 3rd generations would enable farmers to improve the production system based on market demand.

In addition, the maturity being attained by the domesticated *P. pelagicus* in the earthen ponds provides an easy path for broodstock development, which is a vital requisite for the successful commercial development of any potential species in aquaculture. Sexual dimorphism of the domesticated crabs in G1, G2 and G3 was observed at 60 days after from the commencement of rearing with 5.6 and 6.7 % of female berried crabs in G2 and G3, respectively, whereas 17.6 % of female crabs in G1 were 1st identified as berried on day 90 of the rearing period. The present results contrasted Maheswarudu *et al*. [5], who reported some coupling pairs and berried females in the earthen ponds at 120 days of crab rearing, with 32 % of female crabs being sexually mature at harvest in the grow-out pond at 135 days. Oniam *et al*. [23] reported that berried female crabs were first found on day 90 (12.73 % of all female crabs) with a prominent peak on day 150 (38.99 % of all female crabs) of the rearing period. We believe that the contradictory results were due to domestication of the crabs used in the present study. In general, domestication improves the reproductive and growth performances of the domesticated stocks, as has been shown for other crustaceans [14-17,34. The growth rate and sexual performance of the domesticated *P. pelagius* in the present study were consistent with these other results Oniam *et al*. [21] showed clearly the superiority of domesticated female *P. pelagicus* broodstock over wild-caught females. In addition, Trijuno *et al*. [35] reported that the use of domesticated *P. pelagicus* broodstock could improve larval and juvenile crab grow-out performance more than by usinge wild broodstock.

The impact of domestication of *P. pelagius* in the 1st to the 3rd generations was good overall productivity performance of the domesticated stock. However, it is currently unclear whether or not differences observed in other traits, such as effect of inbreeding, might be a result of the short domestication period (only 3 generations) of the crabs in the present study compared to the more than 5 generations of broodstock investigated in the study by Pullin *et al*. [36] where domesticated populations were reported to be susceptible to inbreeding, with potential negative impacts on growth and reproductive performance and traits important for aquaculture.

Based on the present results, the domestication of *P. pelagicus* has good prospects to improve its development as an aquaculture species. The present results suggested that the domesticated stock used in the trials had been properly managed. However, good crab rearing management practices must 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 [37]. A domesticated stock resource is beneficial in reducing operational costs and enabling year-round availability with less seasonal variation [38]. In addition, in other crab species such as the mud crabs, *Scylla serrata* [34], *S. tranquebarica* [39], and the Chinese mitten crab, *Eriocheir sinensis* [40], the selective breeding program from domestication stock resource can improve productivity of these crab. Furthermore, this stock resource is useful for a genetic selection program which can facilitate the commercial culture of this species and this would be an interesting topic for future study to enhance the development of genetic improvement strategies that could lead to the establishment of a selective breeding program that produces the 1st genetically improved strain of *P. pelagius*, possessing desired traits such as fast growth and high survival rates.

Conclusions

This study provided data on the growth rate and sexual performance of the domesticated blue swimming crab, *Portunus pelagicus* in the 1st to the 3rd generations. Growth performance (body weight and specific growth rate) in the domesticated G1 generation was higher than for the G2 and G3 generations. Values for the survival rate, feed conversion ratio and productivity of the crabs reared did not significantly differ among generations. Sexual dimorphism in G1, G2 and G3 was observed at 60 days of rearing, with a sex ratio of 1:1. The percentage composition of yellow, brown and dark gray egg colors of the domesticated crabs reared at 150 days were 20.0 - 25.0, 40.0 - 50.0 and 25.0 - 40.0 %, respectively, of berried female crabs.

These findings provided important information to assist in attaining sustainable *P. pelagicus* production in earthen ponds. However, more complete information for crab rearing management is required based on further research on a selective breeding program to improve the commercial income from this crab.

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