REPRODUCTIVE PERFORMANCE OF INTRASPECIFIC HYBRIDS OF BLUE SWIMMING CRAB (Portunus pelagicus)


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ABSTRACT

High exploitation of the blue swimming crab (Portunus pelagicus) has significantly reduced its wild populations. The domestication process of this species has been started; however, its breeding program has not yet been successful. Therefore, this study aimed to examine the reproduction performance of intraspecific hybrids of blue swimming crab from Sorong (Papua) and Barru (South Sulawesi), Indonesia. P. pelagicus from Sorong has a bigger size, while the crab from Barru has a better reproduction performance, including fecundity and egg diameter. Hybridization was conducted between crabs from Sorong and Barru with different combinations of broodstock, namely: Barru female >< Barru male, (BF>< BM); Barru female >< Sorong male (BF>< SM); and Sorong female >< Barru male (SF>< BM). The results showed no significant difference in the fecundity and egg diameter between the three hybridization trials. The number of successfully spawned broodstock from BF>< BM, BF>< SM, and SF>< BM were 3, 3, and 2 broodstock, respectively. Meanwhile, the mean values of egg fecundity from BF>< BM, BF>< SM, and SF>< BM were 117, 109, and 151 eggs/g BW, respectively. Furthermore, the mean values of broodstock fecundity per crab were 7,797 eggs, 10,103 eggs, and 10,605 eggs, while the mean values of egg diameter were 0.58 mm, 0.57 mm, and 0.62 mm, respectively. In conclusion, the intraspecific hybridization was successfully carried out between the Barru and Sorong crab populations showing no differences in the fecundity values and egg diameter between the three crosses. The successful spawning between female crabs from Sorong and male crabs from Barru was higher than that of female crabs from Barru and male crabs from Sorong.

KEYWORDS: egg diameter; fecundity; hybridization; reproduction performance; Portunus pelagicus

INTRODUCTION

In the last decade, the significant increase in fishing of swimming crabs has put substantial pressure on the crab species’ populations. For example, several studies from different countries reported overexploitation of blue crabs leading to a significant reduction in their wild populations (Mehanna et al., 2013; Kunsook et al., 2014). Abol-Munafi & Azra (2018) stated that the total catch of crab species in 2014 was 1,715,535 and reduced to 1,677,266 tons in 2015, which showed a decreasing trend in total global crab fishing production. In Indonesia, the signs of reduction in wild populations of blue swimming crabs (BSC) are strongly indicated by the reduction in the number and size of caught crabs (Madduppa et al., 2021). As a result, the Ministry of Marine Affairs and Fisheries released the Fishing Act No. 1/2015, revised by Decree number 56/2016 to regulate crab size that can be caught by fishermen (Rahman et al., 2019). If not prevented or managed, the wild population decline will affect the species’ genetic variability, survival rate, and reproduction performance (inbreeding depression) (Teixeira & Huber, 2021). These issues can be prevented through crab aquaculture.
However, no aquaculture technology for swimming crabs has been developed, particularly in the breeding and hatchery aspects (Djunaedi, 2009).

The main problem with crab seedlings in hatchery is the fluctuation and low survival rate of crab seeds (Fujaya et al., 2013). Ikwanuddin et al. (2012a) discovered that the survival rate of crab larvae at zoea 1 phase to megalopa (crab) was 2.7-6.8% while Taufik et al. (2016) reported that from zoea 1 phase to zoea 4, the survival rate was 10.21% Meanwhile, the highest reported survival rate of crab larvae (P. pelagicus) was 23.11% (Efrizal, 2017). The quality of crab larvae can be improved by increasing their genetic quality, which will consequently enhance the heritability of the species’ character and influence the fitness of the introgressed lineage (Twyford & Ennos, 2012). Hybridization is a process to increase the genetic quality of broodstock in cultivation. Sabbir et al. (2017) stated that for cultivation purposes, hybridization could improve heterozygosity and exploit degraded aquatic environments. Hybridization can also be implemented in crustacean species, although limited information is available especially for crabs (Baiduri et al., 2014). This is because the reproductive system in the crustacean is more complicated (Graziani et al., 2003), coupled with limited information on reproduction and spawning control (Benzie et al., 1995).

Moreover, hybridization can be carried out through intraspecific breeding of broodstock having excellent character traits for cultivation purposes or between species with geographic and genetic variations (Lafarga de la Cruz & Gallardo Escárate, 2011). Farmers have long considered the BSC (P. pelagicus) a single stock. However, Lai et al. (2010) revised the BSC systematic taxonomy based on a morphometric study combined with DNA analysis. They discovered that Portunus consisted of four different species distributed in several areas/regions. In another study by Hidayani et al. (2020), BSCs collected from three locations in Indonesia showed a high genetic variation, one of which was Barru BSC (South Sulawesi) population. Fujaya et al. (2019) further stated that genetic variation amplified by phenotypic characters, such as body size, reproductive performance, and reproductive performance, play an essential role in supporting the breeding program and development of P. pelagicus aquaculture in the future.

Oniam et al. (2012) found that the reproductive performance of crabs varies between and within species due to various factors, such as age, size, diet, and ecological conditions. In Indonesia, Hidayani et al. (2018) discovered that BSC from Sahul Shelf (Sorong, Raja Ampat dan Kaimana), West Papua Province has a larger carapace width and faster growth compared with BSC characteristics from Barru waters described in the study by Hidayani et al. (2015). Despite having a smaller carapace size and slower growth, Barru BSC has a better reproduction performance of characters than BSC from Jepara, Maumere, and Raja Ampat. The best reproductive performances of Barru BSC are mainly due to its high egg fecundity with a smaller egg diameter. For aquaculture purposes, hybridization is one of the methods to improve genetic quality and obtain high-quality broodstock and, in a shorter period, increase heterogeneity and dominant genetic variation (Xing et al., 2022).

Information about BSC hybridization of broodstock from different locations in Indonesia has not yet been documented. However, several steps for testing the capability of species to be hybrid include the viability test (hybridizing ability), where the parameters were growth test and survival rate, ratio sex and gonad maturation, reproduction performance, and fertility (Xing et al., 2022). The purpose of this study was to examine the ability of blue swimming crab intraspecies hybridization between Sorong and Barru broodstocks which are expected to have the potential to produce seeds with hybrid characteristics of fast growth and high reproductive performance.

MATERIALS AND METHODS

Preparing Broodstock of Portunus pelagicus and Container

Crab broodstocks were sourced from two districts: Barru (South Sulawesi) and Sorong (West Papua). The BCS from Sorong were delivered by air cargo, while the Barru crabs were taken from the local fishermen and transported using open styrofoam and buckets equipped with an aerator. After arrival, the crabs from Sorong were transported under closed conditions from Hasanuddin Airport to Siddo, Barru district. During transportation, the crab broodstocks from Sorong were supplied with oxygen.

The maintenance containers used three square fiber tubs sized 2 × 2.5 × 6 m and the water level was set at 50 cm deep. Each container was divided into four sections using nylon nets following the treatments to be tested.

Measurement of Length and Width of Carapace and Body Weight

After arriving in Siddo, Barru district, all crabs were measured for carapace length, width, and body weight. Female and male BSC were separated, and their carapace length was measured from the anterior border
to the posterior end of the carapace. The carapace width was measured from the longest end of the spine at the body side of the carapace.

All males and females were measured for their body weight. Gonad weight was measured after the female broodstocks had spawned. They were then spread in the rearing containers. Some crabs stocked in the rearing containers showed mating behavior after three days. Egg samples were collected from the females carrying the eggs and were weighed using a digital scale with an accuracy of 2 decimal places. The egg diameter was measured for 10% of the eggs and fixed with Gilson’s solution to preserve the eggs.

Hybridization Design of BSC

A total of nine crabs which include male and female broodstock from each population of Barru and Sorong were used for the interpopulation reciprocal hybrid. The hybridization between broodstock was arranged as follows: Barru Female >< Sorong Male (BF>< SM), and Sorong Female >< Barru Male (SF>< BM). Barru Female >< Barru Male (BF>< BM) and Sorong Female >< Sorong Male (SF>< SM) were pure-breed. The measured parameters included the number of pairs of broodstock that successfully mate and spawn, fecundity, and egg diameter from the spawned broodstock.

Spawning Method

Each BSC broodstock was tagged for easy identification and was reared in the container. As described previously, each container was divided into four sections using nets and populated with BSC at a density of one pair of crab/net (Oniam et al., 2018). The bottom of the container was filled with sand as a substrate with a thickness of 4 inches. Sand substrates were used for dwelling, protecting, and maintaining the egg position during spawning (Safaie et al., 2013). The container was equipped with aeration, and the water depth was set at 50 cm. A 30-50% water replacement per day was carried out in the morning (Oniam et al., 2012; Ikhwanuddin et al., 2012b). The crab broodstock was fed by trash fish (Selaroides sp.) as much as 10% of body weight (Atifah, 2016) and squid (Loligo sp.) as much as 5% of body weight (Qamariah et al., 2016) twice a day in the morning and the afternoon.

In this study, the crab was spawned naturally and monitored daily. The broodstock was transferred to a cone container as a hatching container with a capacity of 240 L. The hatching container was filled with 200 L seawater at a salinity of 30-32 ppt. Before they were transferred into a cone container/hatching container, the crabs were submerged in formalin solution (1,000 ppm) to get rid of the diseases.

Fecundity

Fecundity is the number of eggs per female broodstock (egg/individual). The egg sample was taken from berried females. The fecundity counting was carried out using a combination method of gravimetric and volumetric (Fujaya et al., 2019).

\[ F = \frac{TGW \times ES}{SGW} \]

where: \( F \) = fecundity
\( TGW = \) total gonad weight
\( SGW = \) sample gonad weight
\( ES = \) number of egg

Afterward, 1 mL of egg that had been homogenized was taken and put into Sedgwick-Rafter to count the egg number under a microscope and repeated three times. The number of eggs was calculated using the formula below:

\[ ES = V \times X \]

where: \( ES = \) number of eggs
\( V = \) dilution volume (mL)
\( X = \) number of eggs per mL

Egg diameter

A total of 100 eggs were taken from the homogenized egg using a binocular microscope and ocular micrometer with an accuracy of 0.1 mm.

Data Analysis

The data collected were tested for normality and homogeneity of variance. If the data did not follow a normal distribution and the variance was not homogeneous, then non-parametric analysis was used. Fecundity data and egg diameter were analyzed using the Kruskal-Wallis test with a further Dunn test due to the data were not normally distributed after the normality and homogeneity tests, so a non-parametric test was carried out which was equivalent to ANOVA. The analysis was carried out using Microsoft Excel 2017 software.

RESULTS AND DISCUSSION

Broodstock Spawning and Fecundity

The sizes of P. pelagicus broodstock between crossed breeding (hybridization) are shown in Table 1, and the number of those that were successfully mating and spawning is presented in Table 2.
In this study, the carapace length and width of the male and female broodstock from Sorong (SM, SF) were rateably balanced with the species from Barru (BF, BM). This indicates that a successful mating is influenced by the size of the male and female broodstock. It also shows a positive correlation, where large-sized females will mate with males equal in size or larger. This phenomenon has also been reported in several decapod species and other crustaceans (Alberts-Hubatsch et al., 2016). The mean of broodstock weight was closely related to that of fecundity both per individual and gram of body weight, shown in Figure 1 and Figure 2, respectively. The results showed that the mean fecundity per individual was between 77,970 and 106,050 eggs, while the mean value of fecundity per gram of body weight was between 1,171 and 1,509.13. Moreover, the highest fecundity value was achieved by the crossed breeding between Sorong female > < Barru male (SF > < BM).

![Figure 1](image.png)

**Figure 1.** Average fecundity (number of eggs) of blue swimming crabs per individual from each crossed breeding. Barru Female > < Barru Male (BF > < BM), Barru Female > < Sorong Male (BF > < SM), and Sorong Female > < Barru Male (SF > < BM).
Approximately 72.2% of the broodstock from Sorong did not survive during the period of adaptation and maintenance. Out of the 18 females of Sorong BSC, only two crabs successfully mated with the males from Barru BSC and spawned but did not hatch the eggs, while only three of the nine Sorong BSC males were crossed with a female crab from Barru. However, the male crabs from Sorong died after mating. For the pure crossed breeding group from Sorong (SF < SM), all the crabs died during rearing because they could not adapt and were stressed by the new environment. However, this research showed that mating was successfully carried out between Barru and Sorong using a balanced body size.

The correlation between successful mating and the size of the male broodstock is related to the male's biological strategy to defend and protect females from the opposite sex (Kalate et al., 2018). Generally, mating in crustaceans, including Portunidae, occur when the female is molting. Therefore, it is very susceptible to predation and requires protection from males (Alberts-Hubatsch et al., 2016). Fazhan et al. (2017) further stated that the protection of males against females during the mating process could be seen from the behavior of males holding females. This protection occurred until the female integument has hardened sufficiently (Waiho et al., 2015). The size of the female also influences the mating process because it is related to the number of sperm transferred to the female. Meanwhile, females with larger body sizes are usually store more sperm and maximize male reproductive success (Rodgers et al., 2011). The female body size also affects fecundity because a large size, especially the width of the stomach, can produce a higher number of eggs (Oniam & Arkronrat, 2014).

The stomach of the female crab plays a role in the protection and hatching of eggs attached to the pleopods, which hold the egg mass and act as an incubation space for egg development (Ribeiro et al., 2013). Fecundity refers to the result of reproduction showing the number of eggs produced by female species, which varies with individual size (Oniam & Arkronrat, 2014). It has been discovered that high fecundity can increase sperm competition in egg fertilization (Rodgers et al., 2011). Meanwhile, the value of egg produced was very low compared to a previous report (Fujaya et al., 2019), where a total of 99,711 and 135,933 eggs produced by broodstock from Raja Ampat and Barru were obtained. In this study, the number of egg produced between 7,797 and 10,605 eggs (Figure 1) were from broodstock whose carapace length and width (Table 1) were almost the same as in the previous reports by Fujaya et al. (2019). However, the weight of female broodstock used in the study by Fujaya et al. (2019) was lower, with a value of 170.08 g and 94.51 g for crabs from Raja Ampat and Barru, respectively. This study's result was also lower compared to the reports by Ikhwanuddin et al. (2012b), where the fecundity was approximately 350,000 eggs using broodstock with a carapace that ranged from 9.74 to 13.32 cm and 75-235 g bodyweight. This study's results was also much lower than the results by Johnson et al. (2010). Furthermore, Johnson et al. (2010) successfully produced...
fecundities of crabs ranging from 730,000 to 1,500,000 using broodstock with a carapace length and body weight ranged between 5.5-7.9 cm and 132-333 g, respectively. This showed that the carapace length of the crab was within the range above (5-6 cm), but with a much lower body weight, which significantly determines the fecundity value when compared to the size of the carapace. Meanwhile, Josileen (2013) discovered that the fecundity value was strongly influenced by carapace size instead of body weight in the BSC. Safaie et al. (2013) also stated that the fecundity produced by females varies based on individual size and between each species of the same size.

Fecundity in each female is significantly determined by the readiness to spawn. This readiness is related to gonad maturity, which varies based on carapace width or body weight (La Sara et al., 2016). Generally, females with a large carapace size and body weight will produce more eggs (Oniam et al., 2012; Ikhwanuddin et al., 2012b) because they have a longer period of accumulating energy reserves needed for eggs production (Safaie et al., 2013). However, based on the fecundity data per unit body weight, the lowest value obtained was from the female Barru broodstock at the BF > < SM crossed breeding. This is related to the development of broodstock for each female. According to Graham et al. (2012), egg-gripping strength decreased in brooders that matured for the second time or more (multiparous) compared to parents that matured for the first time (primiparous). Based on the body size, BF species in the BF > < SM hybridized group had a larger size (multiparous parent) than the BB parent in the BF > < BM group (primiparous parent). This is based on observations in the field showing that the broodstocks from Barru, which are small in size, have spawned to prevent extinction as a result of over fishing. Although the overall fecundity in this study showed a much lower value than others. Generally, the fecundity of crabs ranges from 78,000 to 1,000,000 eggs in Johor, Malaysia (Ikhwanuddin et al., 2012b), 75,000-325,000 eggs in Western Australia, and 250,000-750,000 eggs in India (Johnson et al., 2010). Low fecundity in this study was due to the small number of crabs used, resulting from high mortality during maintenance. Meanwhile, the mean value of egg diameter obtained in this study is almost the same compared to the previous reports (Fujaya et al., 2019).

Eggs Diameter

Similarly, the size of egg diameter in crustaceans varies based on several factors, such as maternal size, season and temperature (Graham et al., 2012). The mean egg diameter value was in the range of 10.28-11.47 μm, which indicated that the crossover SF > < BM also had the highest egg diameter, as indicated in Figure 3.

Figure 3 indicates that the yellow egg diameter’s mean value was 10-11 mm, while the previous study by Fujaya et al. (2019) was 9-11 m. It was also discovered that the egg diameter increased with larger broodstock size, following an inverse relationship with fecundity (Graham et al., 2012). However, the results showed that the SF > < BM group had proportional fecundity and egg diameter values. These values were due to historical differences in nutrition intake from their previous environment. In addition, the possible influence of stress due to adaptation, especially crabs from Sorong, gave a low reproductive performance for both males (sperm quality) and females (egg quality). Several broodstocks from Sorong failed to spawn and died due to stress (the remaining two females and three males survived) because of the adaptation time of only 24 hours. In this study, the parent crab from Sorong required a longer adaptation time (2-3 days) to new conditions in another hatchery environment, affecting both its reproductive output and survival. However, several broodstocks from Sorong successfully mated but took longer time than those of Barru. It is assumed that the successfully spawned crabs from Sorong have the innate ability of the individual to change behavior, physiology, morphology, or life history in response to environmental cues. This was called phenotypic plasticity, which occurs due to the interaction between genes and the environment (Hollander et al., 2014).

Despite a low fecundity value and egg diameter obtained in this study was almost the same as in previous study by Fujaya et al. (2019), the data from the results showed the success of interlocation mating. This indicated that interpopulation hybridization was assumed to produce heterosis (increased fitness). Moreover, individual fitness mainly shows that two individuals successfully reproduce. Since several parameters affect reproduction, fitness can also be used to represent all aspects of the biology of the individual concerned that affect reproduction (Gardner, 1997), such as fecundity (Oniam & Arkronrat, 2014) and embryonic development as egg diameter (Hamid et al., 2016). In this study, the expected heterosis was to produce crab offspring with fast growth and high reproductive performance but require an extended period for mating. This study also focused only on the reproductive ability of two individuals with different populations based on fecundity and egg diameter values. According to Kaeppler (2012), the
expression of heterosis in hybridization between populations will separately depend on differences in gene frequency and interactions among these populations, including influences of dominance and epistasis. However, the emergence of heterosis in hybridization is difficult to predict and depends on the stage of fish development (Granier et al., 2011). Based on the results of this study, intraspecific (between locations) hybridization can be carried out by applying routine genetic monitoring to characterize blue swimming crab hybrids that can be used as superior broodstocks that contribute to the development of crab aquaculture in the future. However, it is important to note that broodstocks from remote locations require a longer adaptation in a new cultivation container to increase spawning success.

CONCLUSION

The intraspecific hybridization was successfully carried out between the crab populations of Barru and Sorong and showed no difference in the value of fecundity and egg diameter in the three crosses. It was assumed that the broodstock from distant sources requires a longer adaptation to a new cultivation condition to increase the success of spawning. The mating and spawning success of hybridization between female crabs from female Sorong and male Barru was higher than that of female Barru and male Sorong. Furthermore, fecundity was significantly influenced by body weight, female crab development, and the readiness of the broodstock during spawning. Broodstocks with greater body weight, but multiparous produces lower fecundity than those with smaller body weight, which are primiparous. The readiness of the broodstock can be observed based on the maturity level of the gonads.

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REFERENCES


Reproductive performance of intraspecific hybrids ..... (Andi Aliah Hidayani)


