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Reproductive performance of female blue swimming crab (*Portunus pelagicus*) from some waters in South Sulawesi

Andi Aliah Hidayani¹, Yushinta Fujaya¹, Muh. Tauhid Umar¹, Wilda¹, Gardenia Wahab¹, Ani Yuliana¹ and Andi Ivo Asphama²

¹Fisheries Department, Faculty of Marine Science and Fisheries, Hasanuddin University, Makassar, Indonesia

²Makassar Nusantara Polytechnic

Email: Yushinta.fmuskar@gmail.com

Abstract. The blue swimming crab (*Portunus pelagicus*) is one of the endangered species due to overexploitation. The development of hatchery technology is expected to be a solution in overcoming this problem. However, until now the availability of seeds is not stable due to high mortality, especially in the larval stage which is often caused by poor quality of broodstock. This study aims to assess the reproductive performance of small crab crabs from various small crab production centers in South Sulawesi to find quality broodstock sources. The sampling locations included 3 areas, namely Bone Regency, Maros Regency and Takalar Regency, South Sulawesi Province, Indonesia. The reproductive performance measured was the length and width of the carapace as well as body weight, fecundity, egg diameter and number of larvae produced by each broodstock and assessing the development of egg diameter based on embryo development. The results obtained, namely the value of fecundity, egg diameter and number of larvae were different from each region. The highest body size, fecundity, egg diameter and embryo development and number of larvae were obtained from Takalar while the lowest was from Maros ($P < 0.05$). The aquatic environment affects reproductive performance. This can be seen from the infestation of ootolasmis and barnacles found in nearly 90% of bone broodstock which is thought to have an effect on reproductive performance. From this research, it is obtained an illustration that the broodstock of Takalar is better used as the source of broodstock.

1. Introduction

The results of research from a number of countries report that the population of small crab crabs in nature is decreasing due to overexploitation [1,2]. In Indonesia, the highest exploitation areas for small crabs and crabs are East Java, South Sulawesi and North Sumatra [3]. In South Sulawesi, crab exports experienced a decline in the January-May 2019 period, which only reached 199,343 kg from 404,380 kg in the January-May period 2018 [4]. This overexploitation results in fishery depending on seeds from nature for cultivation purposes [5]. Crab cultivation is one solution to meet the increasing demand for crab meat and to reduce the negative effects of overexploitation [6]. Crab cultivation activities have been carried out but still have some obstacles, especially in the crab hatchery process. Until now, seed availability is unstable due to high mortality, especially in the larval stage which is often caused by poor quality of broodstock [7].

In order to develop sustainable seed production and to increase the stock of small crab crabs, cultivation techniques must be diversified. Recent studies on portunid crabs have focused on



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increasing broodstock and raising larvae [8], so there is a need for a better understanding of broodstock reproductive performance that directly benefits fisheries management and sustainable *P. pelagicus* cultivation [9].

Knowledge of the reproductive performance of a species is one of the most important aspects in analyzing harvesting strategies for exploited populations [10,11], and can also be used in stock assessments and developing and evaluating fisheries management strategies [12]. In addition, it can also be used as a basis in the seedling process [13]. Studies of crab reproductive performance have been carried out by several researchers in other countries [5,10,14–18] and also in Indonesia [11,19–21].

Research on reported reproductive performance shows variations in results obtained based on regional differences [9–11,22]. In Indonesia, information related to female reproductive performance, especially in South Sulawesi, Indonesia, is still very limited, including by [23] in Pangkep district. Therefore, in this study, analysis of local data related to the reproductive performance of female crabs from three locations in South Sulawesi was carried out with the aim of determining the source of quality broodstock to support sustainable crab hatchery. In this study focused on the reproductive performance of females, especially those related to morphometrics, fecundity, egg diameter and the number of larvae produced by the mother from three locations in South Sulawesi, namely Bone district, Maros district and Takalar district.

In Indonesia, information related to female reproductive performance, especially in South Sulawesi, is still very limited. Therefore, the aim of this study was to analyze the reproductive performance of small crab crab from three crab production centers, namely Bone, Maros, and Takalar districts to determine the source of quality broodstock to support the crab hatchery in a sustainable manner. The reproductive performance which is the target in this study is fecundity, egg diameter and the number of larvae produced by the mother from the three locations).

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2. Material and methods

2.1. Research location and sample collection

Sampling was carried out in three water areas located in three small crab production centers, namely Bone district, Maros district and Takalar district. The sampling location is illustrated in Figure 1.

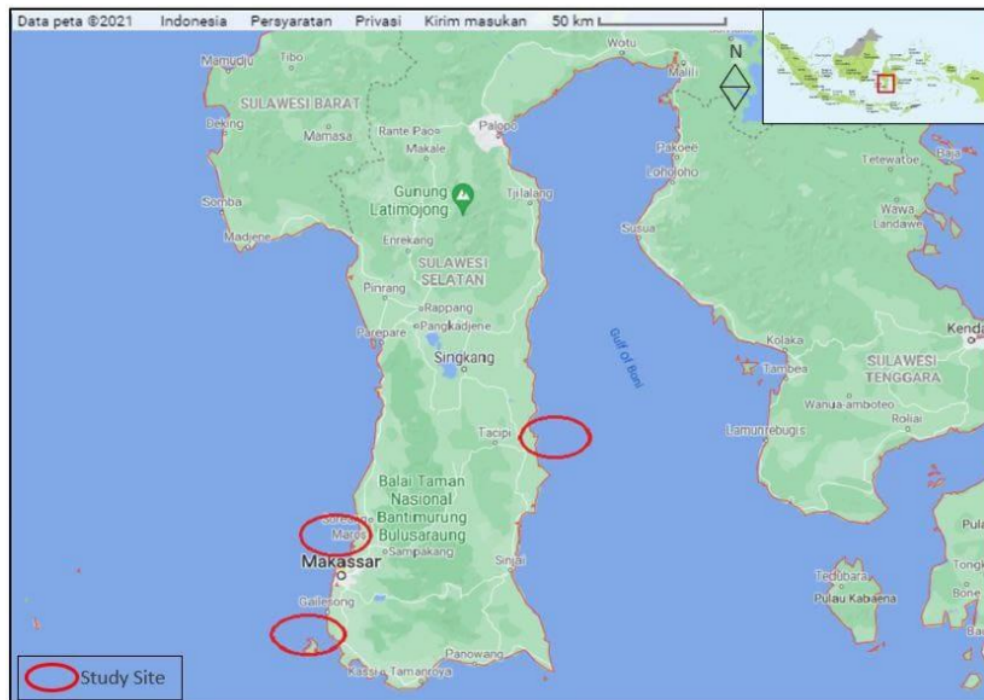


Figure 1. Blue Swimming Crab sampling location.

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A total of 40 crabs carrying eggs were collected from each region. The crab samples were grouped based on egg color, namely 10 each for crabs with orange, brownish and blackish yellow eggs. 10 other tails with blackish eggs are used for hatching. Each crab sample collected was weighed in situ using a digital scale with an accuracy of 0.01 g and the length and width of the carapace were measured (Figure 2) using a calipper.

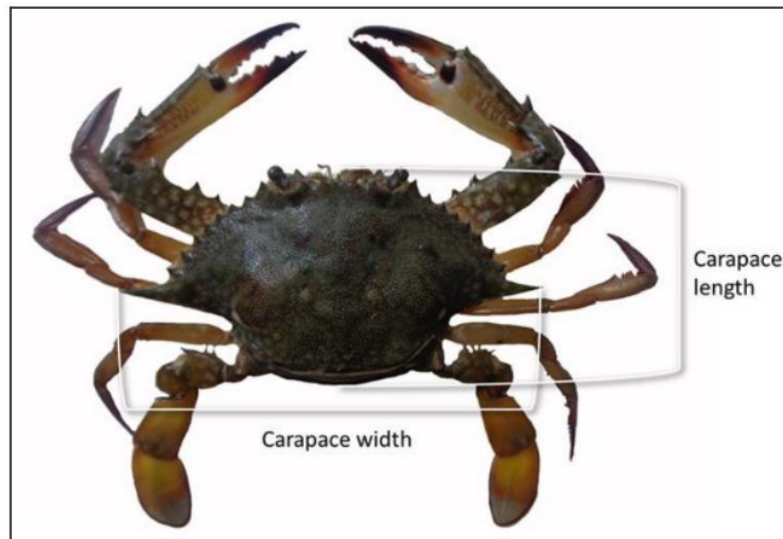


Figure 2. Measurement of carapace length and width of *P. pelagicus* (female) [21].

2.2. Egg collection

Egg collection is done by removing all the eggs that are under the crab's abdomen and then weighing them. Furthermore, 10% of the eggs are set aside and stored in bottles that have been filled with 20 mL Gilson's solution. The sample is stored until measurement is taken.

2.3. Measurement of reproduction performance

The reproductive performance to be measured is fecundity, egg diameter, and the number of larvae that have successfully hatched.

2.3.1. Fecundity. Fecundity is the number of eggs per female broodstock (grain). Fecundity was calculated using a combination of gravimetric and volumetric methods [21]. Egg samples amounting to 10% of the total eggs that have been collected are diluted to reach a volume of 100mL. Furthermore, 1 ml of homogenized eggs were taken and placed in the Sedgwick-Rafter Chamber (SRC) to count the number of eggs under a microscope. The counting of eggs on the SRC was carried out 3 times. The number of eggs in the egg sample (10%) was calculated using the formula $ES = V \times X$, where: ES = number of eggs; samples; V = dilution volume (ml); X = average number of eggs per ml. Fecundity is calculated by the formula, namely $F = TGW / SGW \times ES$, where: F = fecundity; TGW = total gonad weight; SGW = gonad sample weight (egg weight 10% TGW); ES = number of sample eggs.

2.3.2. Egg diameter. A total of 100 eggs per sample were observed under a microscope to measure egg diameter. Measurement of egg diameter was carried out using an ocular micrometer with an accuracy of $0.1 \mu\text{m}$ which is attached to a microscope. Observations were made with an enlargement of 40×10 . Determination of the egg diameter was calculated using the formula $(D + d) / 2$ where D is the longest diameter and d is the shortest diameter of the measured egg.

2.3.3. Total larvae. Main crabs holding black eggs are each put in a container containing 7 liters of water, equipped with aeration. After the eggs hatch, the broodstock is then transferred to another container. The number of larvae (zoea) was counted by sampling using a 100 ml measuring cup.

Sampling of 100 mL was repeated 3 times. Zoea in a measuring cup is homogenized and then taken with a 1 mL pipette. The amount of zoea in 1 mL is calculated. This calculation was also repeated 3 times and then averaged to determine the amount of zoea per mL. The total amount of zoea is the amount of zoea in 1 mL times the volume of the hatchery container.

2.4. Data analysis

Fecundity, egg diameter, and number of larvae were analyzed using the Kruskal-Wallis test with a significance level of 5%. Data are displayed with mean ± standard deviation. All statistical analyzes were performed using Microsoft Excel 2017 and using SPSS (version 16.0).

3. Results

Samples from each study location had size variations in carapace length and width and body weight (Table 1).

Table 1. Size of the broodstock (mean ± standard deviation) from the sampling locations.

Sampling location	Carapace length (cm)	Carapace width (cm)	Total body weight (g)
Bone	5.89 ± 1.11	12.42 ± 1.25	117.77 ± 36.92
Maros	5.23 ± 0.73	11.59 ± 1.27	99.57 ± 38.96
Takalar	6.34 ± 0.52	13.02 ± 1.20	123.53 ± 29.88

In this study, the length and width of the carapace as well as the body weight of the female broodstock from Takalar showed the highest number and the lowest was the female broodstock from Maros. The average body weight of the female broodstock is related to the average fecundity produced.

3.1. Fecundity

The fecundity measured in this study was the relative fecundity (fecundity per gram of body weight) in black eggs (Figure 3).

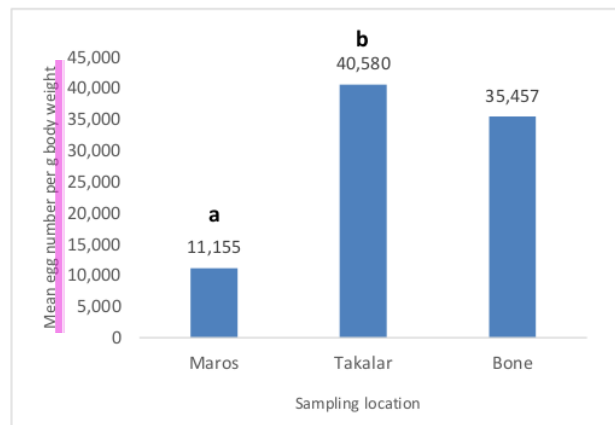


Figure 3. Average fecundity /gram body weight of Portunus pelagicus; different letters above the bar indicate significant differences (P < 0.05).

The results of this study showed that the highest mean fecundity /gram of body weight was the female broodstock from Takalar as many as 40,580 ± 9,120 (mean ± standard deviation), while the lowest fecundity was observed in the female broodstock sample from Maros (11,155 ± 10,697).

3.2. Egg diameter

Not much different from the average fecundity results from each sampling location, the mean egg diameter based on the incubation time with the highest orange, brown and black characteristics also came from the Takalar female broodstock, while the lowest was also from Maros (Figure 4).

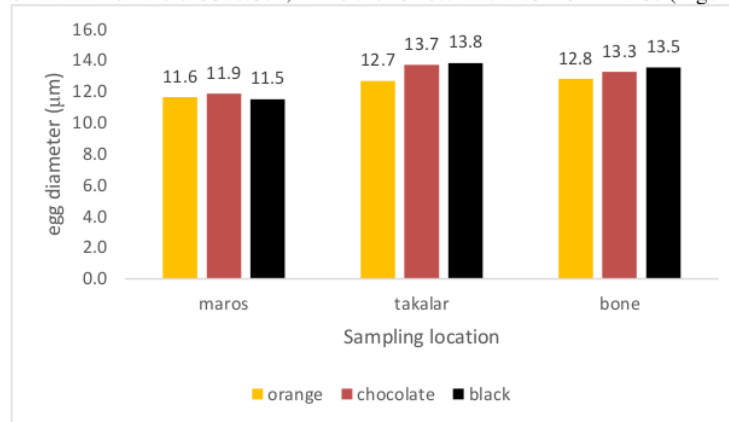


Figure 4. Mean egg diameter of *Portunus pelagicus* incubation time from each location; different letters above the bar indicate significant differences ($P < 0.05$). 16

3.3. Total larvae

The number of larvae produced from each of the 10 crabs from each location shows a varied number (Figure 5). Based on the results obtained, it also shows that the highest number of larvae comes from the Takalar female broodstock and the lowest comes from Maros.

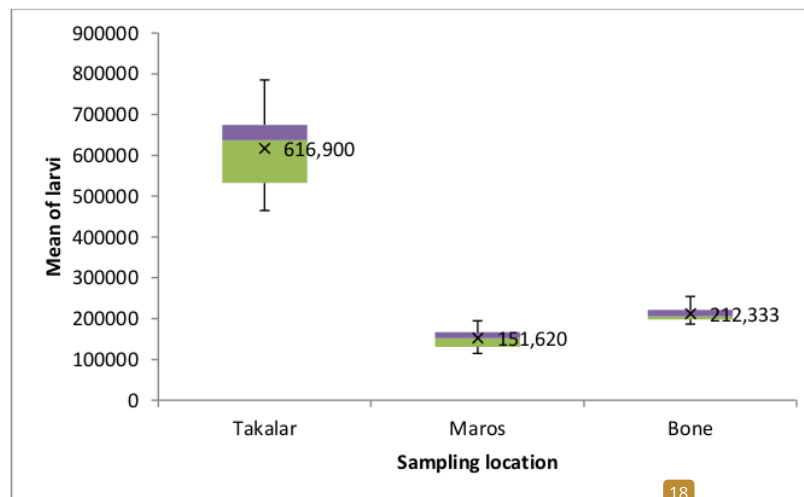


Figure 5. Average number of *Portunus pelagicus* larvae from each location; different letters above the line indicate significant differences ($P < 0.05$). 18

4. Results

Fecundity [12] is an idea of the productivity of an animal. Fecundity is one aspect of reproduction that shows the number of eggs produced by females during the spawning process [12,17,24]. The results showed that the fecundity produced varied from one location to another, where the highest fecundity was obtained from the female broodstock from Takalar and the lowest from Maros. This is in accordance with body size, where the female broodstock from Takalar has the body size of both carapace length and width as well as the highest body weight compared to the other two regions. Some research results also show that fecundity depends on the size of the female body in *P. pelagicus* [10–12,17,25]. According to [26], fecundity will increase up to 83.9% along with the increase in body size, especially carapace width from 105 to 125 mm, because a larger carapace width will be followed by a longer intermoult period so that the time needed to accumulate energy supply time is required for more egg development. In addition, the wider the carapace, the larger the size of the body cavity and the greater the capacity of the pleopods to accommodate a larger number of eggs [11]. Although the fecundity obtained from the female Maros broodstock was the lowest ($11,155 \pm 10,697$), this result was much higher when compared to the female broodstock from Barru [21] who only obtained 1,897 relative fecundity with a range of body weight measurements, almost the same. Barru Regency is also a part of South Sulawesi province.

The existence of fecundity variations of the same species in different areas in the same area is influenced by 2 factors, namely intrinsic factors and extrinsic factors. The main intrinsic factors are variations in the size of individual females or maternal size, nutritional history related to the availability and quality of food, age and age at sexual maturity or first reproduction. The main extrinsic factors include inter and intra-specific competition. Stress to the environment and toxic substances such as differences in biology and genetic composition of each individual as well as parasitism will affect body size [11,27] which in turn will have an indirect effect on individual fecundity.

The highest fecundity was obtained by several researchers from the size of the crab that varied between 120-180 mm [11,26,27]. However, after the main carapace width exceeds 156 mm, the resulting fecundity is highest but less abundant because it contributes a little to total egg production in the population [10,11]. This is because the abundance of initial fecundity increases with the increase in carapace width until it reaches its maximum point and after it decreases [17]. According to [28] and [11] the total abundance of crab fecundity was obtained with carapace widths ranging from 120-140 mm. This is thought to be one of the causes of the low fecundity of the Maros broodstock because the main carapace is only 115.9 mm wide. In contrast to fecundity measurements that are only carried out on black eggs, egg diameter measurements are measured based on egg color. We measured the egg diameter on 3 egg colors, namely orange, brown and black.

The color and size of these eggs indicate the maturity of the embryo. The color of the eggs will change from orange yellow at the first spawning to blackish on the eighth day before hatching [22]. [17] stated that the first day of spawning, the eggs were yellow orange on the second day and dark brown on the third day. Furthermore, it will turn gray and finally black during the last two days before hatching [29]. This change occurs due to the absorption of egg yolk from the yellow egg to a brown color, while the change from brown to blackish gray is due to the appearance of the eyes and pigment spots followed by the abdominal line and cephalothorax [14]. According to Nitiratsuwan *et al.* [30], egg color can also be used as an indicator in determining hatching time, namely 7 days for dark yellow or orange eggs, 6 days for yellow, 5 days for brown, 3 days for blackish brown and 1 day for black gray.

The results of this study indicate that egg diameter increases with time and changes in egg color. [5,14,17], also suggested that generally egg diameter will increase along with embryo development. This is caused by the continuous absorption of water due to respiration and fat metabolism during larval development, or caused by the growth of the embryo in the shell and resulting in forced expansion of the shell which is called the "plastic response" [5,22].

The highest mean egg diameter values obtained in this study were in the range of 12.7 to 13.8 from the broodstock originating from Takalar (Figure 4), while the lowest average egg diameter value ranged from 11.5 to 11.9 obtained in broodstocks from Maros waters. Decapod egg size is one of the factors that play a role in determining the content in the egg itself, where the main components of eggs are more protein and fat. These fats play an important role in reproduction, egg survival and embryonic development, egg hatching and the survival of decapod larvae [22]. The content of fatty acids and protein in eggs is strongly influenced by the feed they get in their habitat [17,31].

What's interesting about the diameter of this egg is that the black eggs from Maros show a smaller diameter than the brown eggs. This is probably the reason why the eggs of the Maros broodstock are the fewest. The highest mean number of larvae was also obtained from the broodstock originating from Takalar. This provides an indication that the fishing area cannot be separated from the aquatic environment. In this study, the salinity data obtained from Maros was 37 ppt. This salinity is higher than other areas, namely 33-35 ppt for Takalar waters and 35 ppt for Bone waters. According to [22], salinity is one of the factors that play a role in the rate of water absorption, and during the late stages of embryonic development, water absorption is complete to support the hatching of *P. pelagicus* eggs.

The effect of salinity on water absorption in embryonic development has been carried out by [17] and show the results that yellow and brown eggs incubated in a salinity of 35 ppt have a larger diameter compared to eggs of the same color incubated at a salinity of 25 and 30 ppt, while black eggs incubated in a salinity of 30 ppt have a diameter greater when compared with black eggs incubated at a salinity of 25 and 35 ppt. This explains that high salinity in the early stages of embryonic development (yellow and brown eggs) can reduce water absorption because they have a thicker membrane compared to eggs that are close to hatching time (black color). With thick walls it can be a barrier to water absorption so that the eggs can still develop properly.

Apart from playing a role in embryo incubation, water absorption is also considered as the start of the hatching process. In the hatching process, the natural swelling of the egg is followed by osmotic swelling of the inner egg membrane, which damages the chorion [5]. The hatching process is highly influenced by water quality, where optimal water quality and maintenance in accordance with environmental conditions can result in a high hatching rate. The embryo may take up water which causes the egg to swell and its membranes to break [17].

Parasite contamination in broodstock also affects the number of larvae. The existence of parasites in the form of ootocysts and barnacles is an indicator that the linkage has a high content of organic matter. This is found in the broodstock from Bone waters. Some of the broodstock caught by fishermen are mostly infected by barnacles because this parasite is an organism that requires a substrate for attachment while the crab carapace is an example of a substrate with a hard surface that can be colonized by benthic invertebrates including the parasite *Octolasmis* sp. Where the carapace of the crab is a temporary substrate for barnacles which is the ultimate goal of *Octolasmis* sp. prior to the gills.

5. Conclusion

The conclusion from the results of this study is that reproductive performance is strongly influenced by the environment in which the crabs live. Water conditions affect the rate of water absorption prior to hatching which is indicated by an increase in the diameter of the eggs. Lack of water absorption at the end of embryonic development will affect the hatch rate. In addition, contamination of nuisance animals such as ootocysts and barnacles also affects reproductive performance. In this study, Takalar was one of the main source areas for female crabs that had the highest reproductive performance.

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