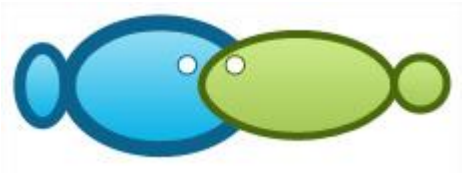

Selection response and reproduction performance of selected giant freshwater prawn (*Macrobrachium rosenbergii*)

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Abstract. High productivity and good performance of the harvested prawn is the main goal of the intensive culture of the giant freshwater prawn (GFP). The prawn size influences its market price, where the bigger prawn has a higher price. The purpose of this study was to evaluate the selection response and reproduction performance of two generations of selected prawn. The selection was conducted by simultaneous mass selection method, consist of traits of body standard length and maturation stage of the GFP female, on the GIMacro (Genetic Improvement of the *Macrobrachium rosenbergii*) strain. Selective breeding to produce faster growth and later maturation in GFP was done by selection for two generations to obtain 20 percent of the bigger size and lower maturation stage for female prawns as the broodstock candidates on the next generation. The result of this study showed that the selection response on growth trait of the GFP was moderate, with 9.68% and 7.77% for the first-generation (G1) and the second-generation (G2), respectively. The bigger selection response was obtained on the maturation trait of the female GFP, with 31.25% and 46.15% for G1 and G2, respectively. The proportion of male morphotype of the harvested prawn was relatively similar, with 46%, 40%, and 47% for G0, G1 and G2, respectively. The result suggests that the simultaneous selection on standar lenght and sexual maturation stage of the female is an effective method to improve the productivity of the GFP.
Key Words: growth, *Macrobrachium rosenbergii*, maturity, reproduction, selection response.

Introduction. The giant freshwater prawn (GFP) (*Macrobrachium rosenbergii*), which is sometimes called "baby lobster", is the most important of the freshwater crustaceans in Indonesia. This species is the biggest freshwater prawn (Ling 1969) with several potential characters, including fast growth which allows them to reach marketable size in about six months and can easily adapt with a wide range of salinity (Chand et al 2015). The omnivorous habit has made the species an excellent candidate for aquaculture (Huawei et al 2020), especially for rural aquaculture (Phuong et al 2006; Wahab et al 2012). Since it's most successful domestication, the culture of freshwater prawn has gained great popularity worldwide, especially in the tropics and subtropical regions (Ling 1969; New & Nair 2012; Banu & Cristianus 2016). Nevertheless, the productivity of the GFP culture in Indonesia is still low and it is caused by the early maturity of female prawn and smaller size of harvested prawn. It is important to solve these GFP culture problems, and a superior strain of the prawn seeds is urgently needed, especially for faster growth and later maturity.

Selective breeding is one of the most effective methods of improving production traits in farmed animals and plants (Gjedrem 1997; Fjalestad et al 2003; Gjedrem & Robinson 2014). Selective breeding for the aquaculture industry has been used to improve several important traits that reduce the cost of production, produces a high-quality product, satisfies consumer preferences, improves fish welfare, reduces stress, and increases disease resistance (Davis & Hetzel 2000; Doupe & Lymbery 2003; Gjedrem & Baranski 2009; Gjedrem et al 2012; Gjedrem 2017). Favourable responses to selective breeding regarding improved production of GFP were reported in several countries, such as Vietnam (Luan et al 2012), India (Pillai et al 2017), Indonesia (Khasani et al 2018), and China (Luan et al 2014; Sui et al 2019).

The productivity value of GFP farming is influenced by several aspects, including harvested prawn biomass, prawn size, and condition of harvested prawn. Commonly, the prawn size determines its price for both on the local and export market, which the smaller is cheaper than the bigger prawn (New & Kutty 2010). In related to this matter, the GFP farming in Indonesia was faced to the problem of early gonad maturity of the female prawn, generally at 5-6 months old (Khasani et al 2010; Wijaya et al 2020) comparing to the Thailand GFP which matures at 7-8 months old (Kitcharoen et al 2010). A mature female will grow slower than its immature counterpart (Ra'anan et al 1991). The somatic weight of the mature female will decrease as resources are routed into gonadal development and gamete production (Taranger et al 2010). Furthermore, Khasani et al (2010) stated that most of the prawn eggs will release from the ovigerous female during harvesting and affect the total biomass of the prawn. Therefore, several efforts have been made to prevent early maturity of the Indonesia GFP, including by selection method.

Refer to Gjerde (1984) and Gjedrem (2000), selecting strains with genetically high age and size at puberty has been successfully applied in Atlantic salmon farming, and these traits have been further improved after several generations of selective breeding based on family selection, individual selection, or combinations of these two approaches. Based on this information, the GFP selective breeding to improve growth character and delaying female maturation was conducted to increase prawn farming productivity. The main objective in the present experiment was to study the selection response and reproduction performance of the selected GFP, being one of the first studies on this matter in Indonesia.

Material and Method

Description of the study sites. This study was conducted from 2018 to 2019 at the Research Institute for Fish Breeding, Subang, West Java, Indonesia.

Description of the biological material. GI Macro II (Genetic Improvement of *Macrobrachium rosenbergii*) strain, consisting of 400 female and 300 male prawns, was used to produce a base population (G0) in the selection program. The GIMacro II is a superior prawn strain, especially on growth character that was resulted from individual selection and released in 2014 (Krettiawan et al 2014).

Selection method. Mass selection was done every year for 2 years, focusing on growth and maturation stage traits to compose selected prawn population, namely first-generation (G1) and second-generation (G2). The selection was conducted on six months old female prawn population to produce selected and control lines of broodstock candidates. The male prawns used for G1 and G2 consist of blue claw and orange claw males, both for selection and control line. The female broodstock candidates of selected and control population (six months old) were used to produce G1 and is shown in Table 1. The female and male broodstock prawns of both selected and control lines were separately reared for 30 days in several cement concrete ponds to improve their size and maturity level by low- rearing density and high protein (38%) of given feed.

Table 1

The average standard length and body weight of selected and control line of G0 female broodstock candidates to produce G1

Population	*Number*		*Standard Length*	*Body Weight*
	Female	*Male*	*mm*	*G*
Selected	220	100	79.00±3.00	21.56±1.46
Control	506	175	72.71±0.98	18.05±1.91
Selection differential			6.29	3.50
Selection differential (%)			8.6	19.30

Mass selection was applied in this study by selecting 20% of the best performance female GFP based on standard length (SL) of prawn body and continued to select 50% of the lowest maturation stage of female GFP. The maturation stage of female GFP was classified into five stages. Figure 1 depicts the stages in the following manner: stage 1 shows an immature female, with the ovary being without exogenous vitellogenic oocytes and with a narrow egg chamber; stage 2 shows a mature female, with the ovary showing yellow to dark green filling of vitellogenic fluid; stage 3 shows an ovigerous female with orange eggs; stage 4 shows an ovigerous female with grey eggs; stage 5 shows a spent female with opened brood chamber (Sagi & Ra'anan 1985; Revathi et al 2012).

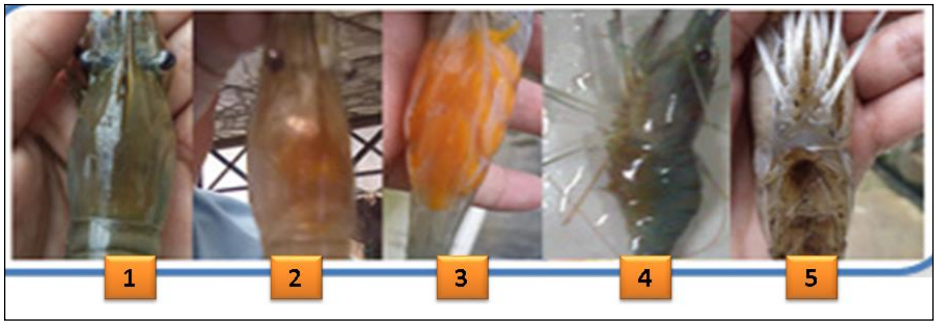

Figure 1. The maturation stages of female GFP: (1) immature females; (2) mature female, ovary filled by vitellogenin; (3) female with orange egg (OE); (4) female with grey egg (GE); (5) spent female (SF).

The G1 was composed through communal mating of 220 female and 100 male prawns from selected broodstock of the G0 population. The G2 population was composed through matting selected broodstock from the previous generation which consists of 450 females and 300 male prawns. The prawn matting was conducted in 200 m^2 of concrete ponds for 20 days. The ovigerous females were transferred into incubation tanks to produce prawn larvae.

Larvae rearing was conducted in a clear water system, using 50 larvae/L of initial density. Larvae fed with brine shrimp (*Artemia* spp.) for 10 first days and combined with egg custard until 10 days after reaching the post-larval (PL) stage. Afterward, PL from each line was transferred to a nursery concrete pond (5 × 5 × 0.8 m^3) for 30 days in concrete ponds, each pond was stocked with 5000 PLs, fed with commercially prawn pellets (contain 38% of crude protein). Juveniles from the nursery phase were reared for 120 days in earthen ponds, fed with commercially prawn pellets (contain 32% of crude protein). Due to sexual dimorphism on prawn growth, prawn sexing was done before selection. Control populations for every generation were created by using 40-60% ranked prawn based on SL character. The simple illustration of GFP selection method was shown in Figure 2.

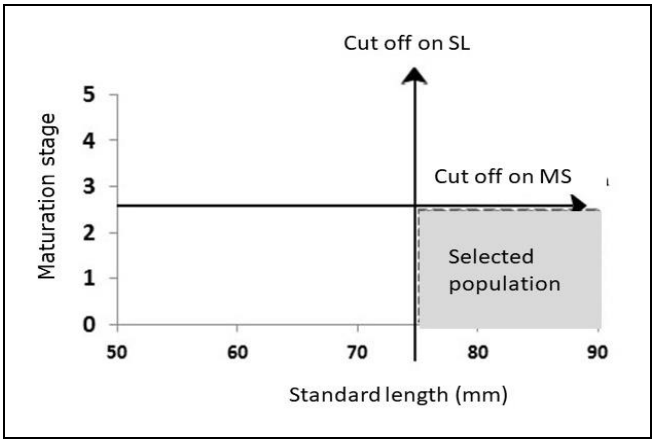

Figure 2. Simultaneously individual selection on growth and maturation stage of the GFP. SL (standard length), MS (maturation stage) (modified from Tave 1995).

Statistical analysis. The selection differential was determined by reducing the average phenotypic data of the selected population with the average phenotypic data of the initial population. Selection response is the average phenotypic data of selected population progeny be reduced with the average phenotypic data of control population progeny (Tave 1995; Gjedrem & Baranski 2009). Heritability was calculated based on the selection response value divided by differential selection (Gjedrem 2005).

$$SD = X_s - X$$

where: SD : Selection differential
X : Phenotypic average of the initial population
X_s : Phenotypic average of the selected population (10% of the top)

$$\mathbf{h}^2 = \frac{SR}{SD}$$

where: h^2 : Heritability
SR : Selection response
SD : Selection differential

Analysis of selection response on maturation trait was calculated based on the percentage of the non-ovigerous female of six months old prawn population. The determining of male morphotype refers to Ra'anan et al (1991).

Results. The simultaneous individual selection of the GFP based on growth and maturation trait is effective to be done. Using 20% of cut off based on standard length to determine selected and control line result in 8,46 mm (12%) and 2,47 g (17%) of the selection differential (SD) for standard length and body weight trait, respectively (Table 2). The total selection response after two generations is low for standard length trait, which is 4,07% (Table 3), moderate for body weight trait, with 17,45% (Table 4), and high for maturation stage, with 77,40% (Table 5).

Table 2
The average of standard length and body weight of the selected and control line of G1 female broodstock candidates to produce G2

Population	*Number*		*Standard Length*	*Body Weight*
	Female	*Male*	*mm*	*g*
Selected	450	300	77.00±0.00	17.32±1.14
Control	516	165	68.54±0.88	14.85±0.65
Selection differential			8.46	2.47
Selection differential (%)			12	17

Table 3
Selection Response (SR) dan real heritability on standard length (SL) trait of individual selection of the giant freshwater prawn

Generation	*Average of SL (mm)*		*SR*		h^2
	Selected	*Control*	*Absolute (mm)*	*Relative (%)*	
G1	69.13	68.13	1.00	1.47	0.16
G2	70.13	68.33	1.80	2.63	0.21
Total of SR			2.80	4.10	
Average			1.40	2.05	0.18
Std dev			0.56	0.82	0.037

Table 4

Selection Response (SR) on body weight (BW) trait of individual selection of the giant freshwater prawn

Generation	*Average of BW (g)*		*SR*		h^2
	Selected	*Control*	*Absolute (g)*	*Relative (%)*	
G1	15.28	13.93	1.35	9.68	0.38
G2	16.82	15.61	1.21	7.77	0.49
Total of SR			2.56	17.45	
Average			1.28	8.72	0.44
Std dev			0.99	1.35	0.0735

The higher proportion of later maturation of prawn females is a goal of this study. The population structure of maturation stage of female in G0, G1, and G2 (Figure 3) showed that the proportion of late maturation female, consist of stage 1 (Immature Females) and stage 2 (mature female, ovary filled by vitellogenin), in the G1 (100%) and G2 (52%) and are higher than the G0 (42%). This means that the selection is effective to reduce proportion of ovigerous female in the harvested prawn. The picture of the male population in the right column shows that the proportion of small male (SM) before selection (G0) and after selection (G2) is not different. This means that the selection in this study has no effect to the proportion of the male prawn.

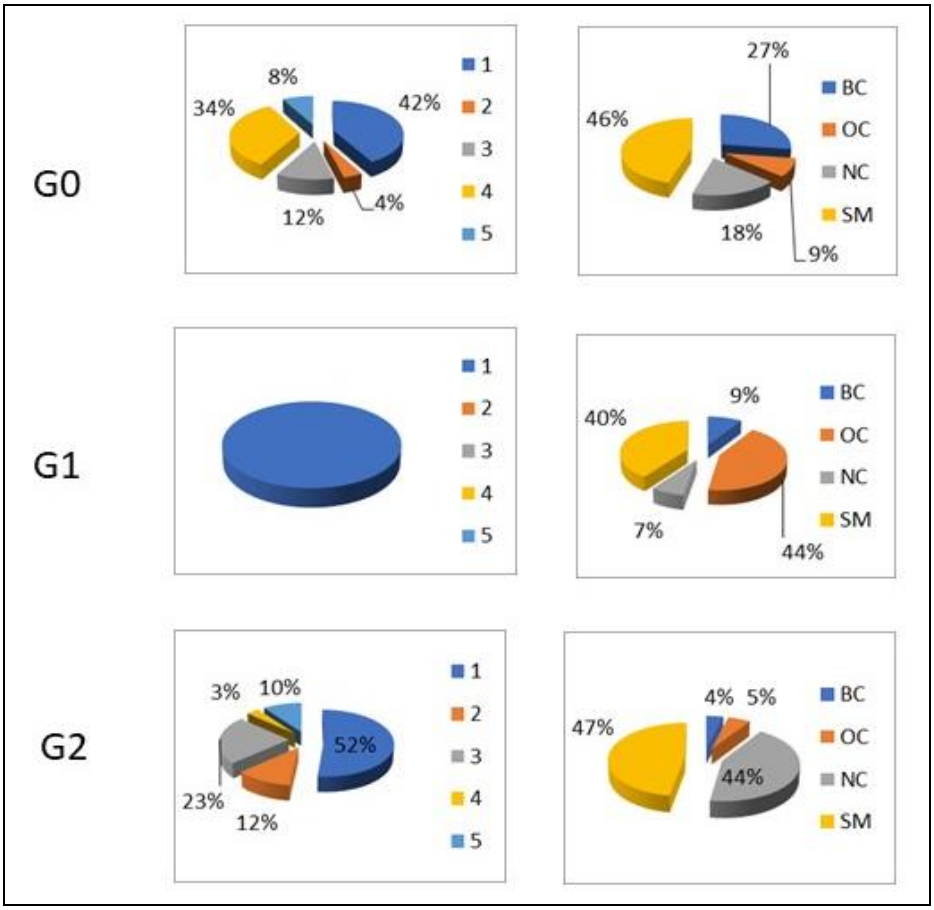

Figure 3. Population structure of female and male prawns in the G0, G1, and G2. (1) Immature Females, (2) mature female, ovary filled by vitellogenin; (3) female with orange egg; (4) female with grey egg and (5) spent female. BC = blue claw, OC = orange claw, NC = no claw and SM = small male).

Table 5

Selection Response (SR) on maturation level trait of individual selection of the giant freshwater prawn

Population	Pre-ovigerouse female (%)		SR	
	Selected	Control	Absolute (%)	Relative (%)
G1	100,00	76,19	23,81	31,25
G2	63,33	43,33	20,00	46,15
Total of SR			43,81	77,40
Average			21.90	38.70
Std dev			2.69	10.53

Discussion. Selective breeding programs can be effective in delaying maturity in farmed fish and thereby also increase body size at puberty (Gjedrem 2000). In the breeding program, the selection response is an indicator of the effectiveness of the method in the fish breeding program by selection. Based on Gjedrem & Rye (2016), the genetic gain of the growth trait for one generation of aquatic animal species has high variance (2-40%), with 12.7% of the average of the genetic gain. Especially for *Macrobrachium rosenbergii* breeding, the selection response of growth was reported as low to high. In Vietnam, the selection response of harvest body weight (HBW) of *M. rosenbergii* reached an average rate of about 7% per generation (Hung et al 2013). In China, it was 6.56% per generation (Luan et al 2012). A previous selection of *M. rosenbergii* in Indonesia achieved an average selection response of HBW of 25,15±15,15% (Khasani et al 2018). In the present study, the average selection response of HBW for two generations was 8.72±1.35% and is higher than the previous study in Vietnam and China, but lower than the previous study in Indonesia. Especially for GFP breeding in Indonesia, the lower selection response of this study than the previous study is caused by the lower selection intensity. The previous breeding used higher selection intensity, we selected 10% of the best performance female GFP (Khasani et al 2018), meanwhile this study selected 20% (lower selection intensity) of the best prawn to produce the next generation. According to Kirpichnokov (1981), the selection response is affected by several factors, including selection intensity, heritability, and standard deviation of the selected trait.

In the present study, the heritability of standard lenght (SL) was categorized into moderate, and was 0.18±0.047. However, the heritability of body weight (BW) was relatively high, i.e. 0.447±0.07. Karplus (2005) mentioned that heritability of size characters in the giant prawns, including total length, cephalothorax length, and claws length was grouped into medium to high and the value was 0.29-0.39 in female population. Meanwhile, the heritability on carcass weight was moderate to high (0.37-0.41) (Hung et al 2013). Furthermore, Kitcharoen et al (2010) said that heritability both on weight and total length of female population of GFP were 0.28±0.17 and 0.47±0.18. The heritability of SL and BW in the previous breeding of the GFP in Indonesia was relativelly higher, and they were 0.48±0.15 and 0.35±0.27 (Khasani et al 2018). The lower real heritability both on SL and BW in this study than the previous study in The Indonesian GFP was suspected by the difference selection intensity which implicate to the selection response.

Besides faster growth, delaying the sexual maturity of the GFP female is a main goal of this study. For several commodities, early puberty is a major problem in farmed fish, such as in salmonids, sea basses, flatfishes, cod fishes, tilapias, sea breams, and perches (Taranger et al 2010). Puberty adversely affects growth, feed utilization, health, and welfare. Gjerde (1984) stated that, when salmonids become sexually mature their growth rate decreases, meat quality is reduced and mortality increases. Early puberty can also increase the risk of negative genetic effects of escapees on wild stocks or after spawning in sea cages (Iversen et al 2016). Therefore, delayed sexual maturation is advantageous for several fish species, including the common carp (*Cyprinus carpio*), the

whitefish peled (*Coregonus peled*), and Tilapia (*Tilapia* spp.) (Kirpichnikov 1981), and for the GFP (Wijaya et al 2020).

In Indonesian GFP farming, early maturity has been observed for one decade (Khasani et al 2010). The problem influences the GFP pond production and the harvested prawn size. The growth of the prawn female will be reduced during egg development since a proportion of the available energy is used for the development of the oocytes (Ra'anan et al 1991; Taranger et al 2010). Refer to Taranger et al (2010), delaying sexual maturation in aquaculture commodities can be conducted by selective breeding for several generations. In the present study, the average selection response of maturity trait was relatively high, there was 38.70±10.53% than the previous studies in several species. The previous studies of selective breeding on maturation trait were reported with positive selection response (Gjedrem & Rye 2016). The selection response of early maturing female in nile tilapia *(Oreochromis niloticus)* was reported at 23.5% per generation (Longalong et al 1999). The selection response in rainbow trout was 6.75% per generation (Sitonen & Gall 1989), and in Atlantic salmon, it was 3% (Gjerde & Korsvoll 1999).

The higher proportion of later maturation in the selected population in G1 and G2 than G0 is a good trend for future selection. Although the difference in the proportion of females between G1 and G2 was high, 48%, the absolute selection response in the two generations were relatively similar, there were 23,81% (G1) and 20,00% (G2). The difference proportion of late mature females between G1 and G2 may be caused by a difference of environmental factors, especially the season and raining rate during the prawn rearing. Refering to Taranger et al (2010), age and size at puberty of aquaculture animals were affected by genetic and environmental factors. On the other hand, the proportion of small males in the G0 and G2 was relatively similar. This means that the selection does not affect to the male morphotype. Kuris et al (1987) and Ra'anan et al (1991) mentioned that the differentiation morphotypes in male prawns is mainly caused by social interaction of the prawn in the mix population. Furthermore, they stated that the proportion of the three male morphotypes in the standard prawn farming system consists of 50% of the small-males, 40% of the orange claw male, and 10% of the blue-claw male. The small males grow slowly and exhibit a normal size distribution. Orange claw males have relatively larger claws, grow rapidly, are highly variable in size. In the special condition, the orange-claw male metamorphoses into blue claw male. Refering to Ra'anan et al (1991), the proportion of small males in the present study is relativelly normal.

Conclusions. Based on the results from the present study, we established a breeding program to improve the growth and delaying the sexual maturity of the giant freshwater prawn female. The average selection response for two generations is moderate for growth trait and high for sexual maturity trait.

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