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Short Communication

Evaluation of Growth Performance and Improving Genetic Gain of Blue Tilapia (*Oreochromis aureus*) Fourth-generation (F-4) at Brackish Water Pond

Adam Robisalmi[1,2,*], Bambang Gunadi[1,2], and Priadi Setyawan[1,2]

[1]Research Institute for Fish Breeding, Patokbeusi Subang, West Java, 41263. Indonesia

[2]Research Center for Fishery, National Research and Innovation Agency, Bogor, West Java, 16915. Indonesia

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*) Corresponding author:
E-mail: aa_salmi@yahoo.com

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Abstract

Breeding program in order to increase genetic improvement in blue tilapia have not been widely carried out at brackish water ponds. This study aimed to evaluate the increase in growth and genetic gain of F-4 blue tilapia from family selection in ponds with 25-30 gL^{-1} salinity. The parent used for the formation of F-4 is the selected parent F-3 and as a control using the non-selected parent F-3. Spawning was carried out in a full-sib mating design using the family selection method. The ratio of male and female broodstock is 1: 2. Spawning and nursery activities of F-4 blue tilapia are carried out in freshwater. The enlargement test was carried out in the net cage 5m x2.5m x1 m which was installed in the ponds with a salinity of 25-35 g l^{-1} for 120 days, at a stocking density of 10 fish m-^{2}. At the end of maintenance, a selection process is carried out on the weight traits. Parameters observed included growth, survival, and genetic values. The results showed that the growth and survival in the F-4 blue tilapia population, male and female selected populations, had a higher value than in the non-selected population. The realized heritability value of the population growth character of the F-4 blue fish is in the high category. The difference in the average weight of the selected blue tilapia and the control was equivalent to an increase in genetic value added by 15.06% (male population) and 17.92% (female population).

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1. Introduction

Tilapia is a fish species that can tolerate various environmental factors including tolerance to low dissolved oxygen and high salinity (Popma and Masser, 1999; Nugon, 2003). According to El-Sayed (2006), several tilapia strains that have high salinity tolerance are blue tilapia (*Oreochromis aureus*), *Tilapia zillii*, *Oreochromis spilurus* and *Oreochromis mossambicus*. In the last two decades, global tilapia aquaculture has been dominated by three species: *Oreochromis niloticus*, *O. mossambicus*, and *O. aureus*. Of these, black tilapia, or *O. niloticus*, is by far the most widely cultivated and accounts for 90% of total tilapia production (Fitzsimmons, 2016). In its development, a cross between black tilapia (*Oreochromis niloticus*) and this species has been carried out where the offspring of *O. aureus* × *O. spilurus* showed the highest growth, while the highest survival rate was for *O. mossambicus* × *O. spilurus* (Tayamen *et al*., 2002; Lutz *et al*., 2010).

Blue tilapia is recognized as a potentially invasive species in most of the temperate and tropical world (Knight and Devi, 2009). This fish has a tolerance at low temperatures of 3-13°C (Kamel *et al*., 2008) and a higher tolerance for salinity above 35 gL^{-1} than black tilapia (Nugon, 2003; Armas-Rosales, 2006), and grows well in the salinity range of 36- 44 gL-1 (Balarin and Haller, 1982). Setyawan (2014) reported that selected blue tilapia G0 can survive at 57 gL^{-1} salinity with 60% survival despite experiencing stunted growth. In contrast to the results of the study by Küçük *et al*. (2013), it was shown that the optimum condition for rearing blue tilapia in brackish water was at a salinity of 12-24 gL^{-1}.

Efforts to increase genetics in tilapia through breeding methods have been carried out in Indonesia, including through hybridization and selection. However, these activities are mostly carried out in fresh waters such as ponds and lakes. There is a concern that selected genetic characters that are enhanced in favorable environments, such as freshwater or low salinity, may not perform well in less conducive culture systems, such as high salinity. Several studies have reported that the response to environmental stressors has not been well documented for the genetic enhancement of tilapia strains. Moorman *et al*. (2014) stated that the sub-optimal environment used for rearing tilapia may have a negative impact on growth performance, survival rate, meat quality and appearance. Likewise, tilapia selection activities carried out in brackish water have not been widely reported, especially in blue tilapia. Gjedrem (2000) stated that the genetic improvement achieved in fish breeding programs is generally 5-20%. Several previous studies reported that the percentage of genetic gain in tilapia ranged from 10 to 13.3% (Bolivar and Newkirk, 2002; Gall and Bakar, 2002; Ponzoni *et al*., 2005). The results of research on the selection of black tilapia in the brackish water of 15-20 gL^{-1} reported by Ninh *et al*. (2014) that after 5 generations showed a genetic increase of about 35% of growth characters.

The family selection program for blue tilapia (*Oreochromis aureus*) conducted by the Research Institute for Fish Breeding (RIFB) is a series of activities in the context of establishing high salinity tolerant tilapia. The results of the study to date have produced a population of third-generation blue tilapia (F-3) as a result of family selection in brackish water ponds with a response to selection of 17.35% (RIFB Technical Report, 2016). This value is higher than the heritability value for the first-generation blue tilapia (F-1) 0.23 with a response to selection of 8.44 %, then in the second generation (F-2) of 0.41 with a response to selection of 14.85% (Gunadi *et al*., 2014; Gunadi *et al*., 2015). The selection activities on blue tilapia in freshwater which were reared for 270 days have been reported by Zak *et al*. (2014) has an estimated heritability value in the fourth generation of 0.58 with a genetic gain of 8.85%.

Based on research data in the third generation (F-3) which shows that there is still an increase in the value of the selection response, selection activities in the next generation (F-4) must be carried out to obtain a population of offspring that have a higher value growth and produce families with higher heredity and response to selection. This research is a follow-up activity that aims to evaluate the increase in growth and genetic gain of the next generation, namely blue tilapia F-4.

2. Materials and Methods

2.1 Materials

This activity was carried out at the Research Institute for Fish Breeding, Sukamandi and brackish water ponds in Cirebon Regency in May-October 2016. The formation of F-4 blue tilapia was carried out using selected F-3 brooders obtained from research results in 2015. In addition, F-4 population formation was carried out using non-selection F-3 broodstock as a control population. The spawning method used was a full-sib mating design with a male and female parent ratio of 1: 2.

2.2 Methods

2.2.1 Spawning

The spawning activity of blue tilapia F-3was carried out in happa measuring 2x1x1 m^3 as many as

80 happa with 3 broods used (1 male and 2 female) in each happa. The total number of families in the fourth generation (F-4) resulting from the offspring selected by F-3 was 26 families. The non-selected F-3 broodstock (control population) produced 10 families. After 10-14 days from the parent plot, then harvest the larvae. The fourth generation (F-4) blue tilapia larvae rearing is carried out separately in each family. The larvae obtained from the set were then put into nursery happa measuring 2 m x2mx1m which was placed in an earthen pond measuring 200 m^2. The stocking density used was 125 fish m^{-2}. During 60 days of nursery, larvae were given commercial feed (38-40% protein) under ad libitum feeding with the frequency of 3 times a day (08.00 am, 11.30 am, and 04.00 pm).

2.2.2 Growing-up stage

Prior to stocking at brackish water ponds, the fourth generation (F-4) blue tilapia juveniles were acclimatized by adding 5 gL^{-1} of seawater which lasted for 5 days. The rearing activities for each family were carried out at brackish water ponds with a salinity of 25 gL^{-1} in net cage measuring 5 mx2.5mx1m . The stocking density applied at this stage was15 fish m^{-2} After the fish reached a body weight (BW) of 10-15 g, a selection was made based on sex between males and females. Enlargement activities are carried out based on sex until the fish reach the size of the prospective broodstock (BW: 150-200 g). Feeding is done 2 times a day (morning and evening) as much as 5-10% of the biomass. The feed given has a protein content ranging from 28 to 30%.

2.2.3 Selection

At the end of rearing in the pond (after 120 days of rearing), then a selection process is carried out on growth characters. The selection process is differentiated by sex between male and female populations. In each family, 50 individuals were randomly sampled and then the weights were measured to obtain size distribution data, which were then sorted from the smallest to the largest values

2.3 Data Collected

Sampling activities for measuring growth are carried out once a month (beginning to the end of maintenance) with the number of samples being observed as much as 10% of the number of fish. Parameters observed were growth including weight gain, growth rate, and survival. The selection activity to evaluate genetic improvement was carried out based on the size distribution data that had been sorted, setting a minimum limit for each family for the size of the fish to be selected, namely 10% of the male population and those with the best phenotypic performances. Based on the minimum limit size that has been obtained, a selection is carried out on the entire population. Prospective male parents are selected based on male size criteria while female parent candidates are selected based on female size criteria. The genetic improvement parameters include the coefficient of variation, selection differential, realized heritability value and response to selection.

2.4 Data Analysis

Statistical analysis to test the weight gain, growth rate, and survival between selected F-4 populations and control F-4 populations. The difference was considered significant at $P < 0.05$ and all data were presented in the form of mean ± standard deviation.

2.4.1 Calculation of the growth performance

The calculation of growth parameter data is carried out based on the following formula. Weight gain is the difference between the final weight with the initial weight of maintenance calculated with the formula ($\Delta W = Wt - W0$) (Zonneveld *et al*., 1991). The specific growth rate (SGR) is the daily growth rate, or the percentage of fish weight added per day, calculated with the formula as follows: SGR = (lnWt-lnW0)/t)*100 based on National Research Council (1977). The coefficient of variation represents the ratio of the standard deviation to the mean, CV = (SD/) * 100 (Singh and Chaudary, 1977). Survival rate and the ratio of the number of fish that live until the end of the maintenance with the number of fish at the beginning of maintenance were calculated using the formula from Effendie (1997).

2.4.2 Calculation of geneticparameters

The calculation of genetic parameters is carried out based on the following formula. The selection differential is the difference of the base population mean (x) and the mean of the selected population (x'), S = x'-x (Singh and Chaudary, 1977). The response to selection is the difference of the control population mean (X_0) and the mean of the selected population (X_1), $R = X_1 - X_0$. The realized heritability is the ratio of response to selection to the selection differential, $h^2 = R/S$, calculated using the formula from Falconer (1981).

3. Results and Discussion

3.1 Growth Performance

Evaluation of growth performance is one of the methods used to see the increase in growth characters, namely intergenerational weight characteristics. In this study, what we want to see is the average of the parents of F-3 blue tilapia on the growth of the resulting chicks, namely F-4 blue tilapia kept in ponds. The body weight between selected and control F-4 blue tilapia (male and female populations) increased every month until the end of the rearing period and there was a difference since the rearing entered the second month (Figure 1). The selected F-4 blue tilapia had a better growth pattern than the control (non-selection) F-4 blue tilapia. The difference in performance between the two populations of blue tilapia is an illustration of the genetic improvement that is passed on from parents to offspring as a result of the selection program. According to Effendie (1997), one of the factors that influence growth is the internal factor. Internal factors include heredity, sex, and age are generally difficult to control. Apart from genetic factors, differences in growth can also be influenced by environmental factors, including nutrition, stocking density, water quality, maternal effects, and co-maintenance (Hargreaves, 2000; Dunham, 2004). Furthermore, these environmental factors will affect the phenotypic appearance of an individual and the fish population that we maintain. This genetic diversity and environmental diversity together form a variety of phenotypes that cause differences in individual appearance (Falconer, 1981).

Growth response testing on F-4 blue tilapia was carried out to evaluate the performance of selected tilapia compared to controls. The results of the calculation of absolute growth and daily growth (Table 1) show that the weight growth of selected F-4 blue tilapia has a higher value than control of 19.94% in the male population and 21.37% in the female population. The value of the specific growth rate of blue tilapia F4 male and female selected populations were 4.90% and 5.18% higher, respectively, than the control population. The high growth value of selected tilapia was accompanied by a lower feed conversion value compared to non-selection of 1.72 (male) and 1.69 (female). The statistical test results of absolute growth values, SGR, DGR, FCR showed a significant difference ($P<0.05$) compared to control, except that the daily growth value of the female population was not significantly different ($P>0.05$).

The average weight gain of blue tilapia F4 male and female selected populations in this study was much better than the average weight growth of F-3 blue tilapia obtained in previous studies of 164.73 g (Robisalmi *et al*., 2016). Although the selected fish had better yields, this indicated a difference in rearing conditions. The main influencing factor is assumed to be salinity fluctuations. The salinity conditions at the time of formation of the F-3 blue tilapia were relatively high, reaching 40 gL^{-1}, while in the maintenance of the F-4 population, salinity fluctuations tended to be stable in the range of 25-30 gL^{-1}. If the environmental conditions applied in the maintenance of the two populations are the same or equivalent, then in theory the performance of the two populations should be the same. This is because the population of F-4 blue tilapia, the non-selection of F-3 broodstock, is a representation of the F-3 population. Gall *et al.* (1993) stated that actual or "realized" phenotypic changes would correspond to the expected response to selection only when environmental influences on parental and progeny generations are identical.

If the comparison is made between populations of blue tilapia F-4 (selected vs control), the difference in growth in the population of blue tilapia selected F-4 with control F-4 indicates an improvement in growth. Likongwe *et al.* (2002) stated that the effects of stressors such as salinity in freshwater fish can affect the osmoregulation mechanism, with an increase in the energy budget for ion regulation (to maintain homeostasis), which consequently affects growth. The effect of salinity on growth can be altered by the concentration of ions, Ca^{2+} and Mg^{2+}, as can non-osmoregulatory effects on metabolism (Watanabe *et al*., 1993). The influence of salinity on the osmoregulation process, namely the activity of Na^{+} gills, K^{+}-ATPase depends on several factors, such as species, strain, body size and temperature (Qiang *et al*., 2013).

The high value of weight gain in the selected population indicates the success of the F-3 blue tilapia selection program which is passed on to its offspring (F-4 blue tilapia) accompanied by better adaptability to salinity compared to non-selection so that the increase in growth is not disturbed. This shows that the body's metabolism is still running well, and a homeostatic process occurs. dos Santos *et al.* (2012) reported that selected tilapia showed faster growth compared to natural populations due to differences in metabolic rates, muscle and adipose tissue growth, and different organ sizes, causing changes in carcass and meat quality. Added by Ariyanto and Listiyowati (2015), the adaptability of fish affects the phenotypic appearance, where strains with broad adaptability will have a relatively good phenotypic appearance in various environmental conditions, while varieties with relatively narrow adaptability will have a phenotypic appearance that is strongly influenced environmental conditions in which the genotype was developed. Villegas (1990) and Armas-Rosales (2006) stated that fish size can affect the salinity tolerance of tilapia, which indicates that larger fish sizes can adapt better than smaller fish.

Table 1. Growth performance parameters (mean ±SD) of selected and control blue tilapia (male and female populations)

Parameter	Male		Female	
	Selected	Control	Selected	Control
Initial weight (g)	2.91±0.39	2.88±0.11	2.86±0.32	2.8±0.22
Final Weight (g)	247.37±19.73[a]	198.59±15.16[b]	210±16.39[a]	165.67±19.43[b]
Coefficient of variation (%)	24.78	20.62	24.52	20.20
Weight gain (g)	244.46±19.66[a]	195.71±15.25[b]	207.14±16.71[a]	162.87±19.39[b]
Specific growth rate (%weight/ days)	3.71±0.11[a]	3.53±0.09[b]	3.58±0.16[a]	3.40±0.11[a]
Daily growth rate (g/days)	2.04±0.16[a]	1.63±0.13[b]	1.73±0.14[a]	1.36±0.16[b]
Feed conversion ratio	1.72±0.041[a]	1.81±0.07[b]	1.69±0.13[a]	1.81±0.40[b]

*Different superscripts in the same column shows that there are significant differences ($p < 0.05$).

Table 2. Heritability and Response to Selection of Blue Tilapia F-4 Male and Female Populations at the end of the 120-day rearing period

Populations	Average of body weight (Populations)		Response to selection		Selection Differential F-3	Realized heritability F-4
	Selected	Control	g	%		
Male	247.37	198.59	48.77	19.72	76.29	0.64
Female	210.00	165.67	44.33	21.11	63.27	0.70

The increase in the genetic value of a population as a result of selection activities is influenced by the value of the coefficient of variation. In fish farming, size differences between individuals are generally related to competition for food, while the coefficient of phenotypic variation in body weight, apart from showing variation in traits, has been used as an indicator of competitive interactions within a population. According to Ninh *et al.* (2011), the value of the coefficient of variation is more influenced by generation and environment, where the higher the value of the coefficient of variation the wider the diversity or the more heterogeneous. It is known that the coefficient of variation in the weight of blue tilapia F-4 in male population selection is 24.78%, 16.75% higher than non-selection (20.62), while the female population has a coefficient of variation of 24.52 %, 17.61% higher than non-selection (Table 1). This value is included in the low category, this is due to the influence of the environment, namely salinity. In general, the coefficient of variation (CV) of live weight *Tilapia tilapia* ranges from 40 to 60%, with a better CV value of 6% when reared in KJA compared to soil ponds (Nguyen *et al*., 2007; Khaw *et al*., 2012; Marjanovic *et al*., 2016). Meanwhile, Nugroho *et al.* (2017) reported that the CV value of selected tilapia from the first generation to the fourth generation ranged from 27.77 to 32.88%, while the third-generation goldfish (F-3) had a CV of 25.24%. Tave (1993) stated that the higher the coefficient of diversity in a population, the more it shows the diversity of individual sizes in the population. An increase in the CV may indicate competition between individuals, conversely, a lower CV, or a decrease in CV, may indicate less competition and a favorable social environment for animals (Jobling, 1995; Adams *et al*., 2000; Mambrini *et al*., 2006).

Survival is a trait that has high economic value in addition to growth in tilapia cultivation, especially in ponds. Several literatures suggest that the genetic relationship between growth and survival depends on the species, environment, and growth stage. Estimates of survival rates at different growth phases will provide basic information for designing breeding programmes. During 120 days of rearing, the selection and control (male and female) F4 blue tilapia died (Figure 2). In male and female populations, the survival rates were 9.19% (male population) and 8.47% (female population) compared to blue tilapia F4 control. More deaths occurred when the salinity of the pond water rose to a level of 30 ppt and was due to the handling process at the time of sampling. In addition, death can occur due to an imbalance of osmotic pressure in the fish body with the aquatic environment. When fish are forced to deal with different salinities, more energy is used to maintain homeostasis than growth. Küçük *et al.* (2013) stated that when osmoregulation is impaired, fish spend more energy retaining sodium and chloride ions in their bodies or releasing them. Fish that survive at high salinity concentrations (30-34 ppt) increase the selection of salinity resistance in tilapia. This is reflected in the high expression of genes (Na+/K+-ATPase -1a and -1b), which are associated with osmoregulation taken from the gills, liver, and kidneys (El-Leithy *et al.*, 2019).

The value in this study is in line with the report by Robisalmi and Setyawan (2014) that the survival of red tilapia populations reared in ponds with 25-40 salinity ranges from 89.50-94.00%. Then black tilapia from individual selection in 15-20 ppt ponds were reported to have moderate survival ranging from 75.30-91.90% (Ninh *et al*., 2014). Likewise, Malik *et al.* (2018) reported that tilapia reared at a salinity concentration of 15 ppt showed a high survival rate of 92%. In addition, survival can be effectively increased through improved livestock, management and feeding practices. Santos *et al.* (2012) showed that a high-protein diet greatly increased the survival rate from stocking to harvesting in tilapia.

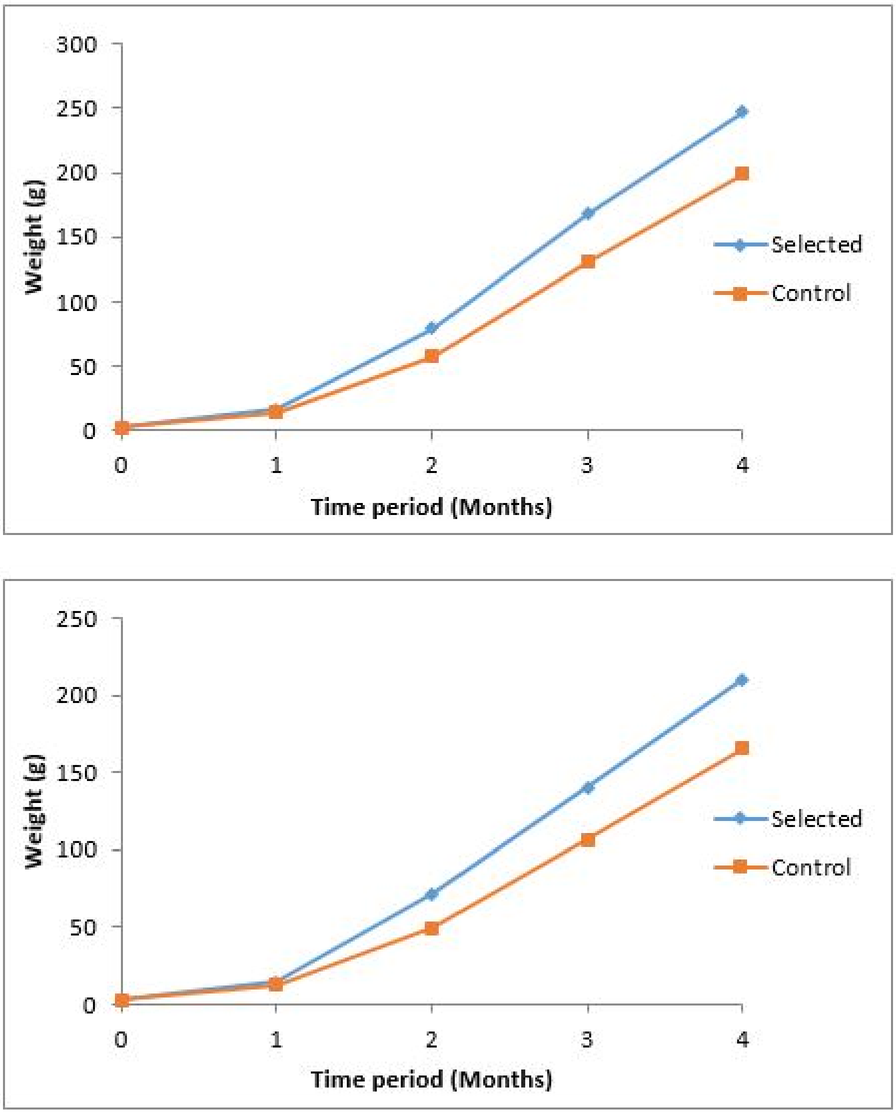

Figure 1. Growth pattern of blue tilapia generation 4 (F-4) male (a) and female (b) population selected and control at brackish water pond

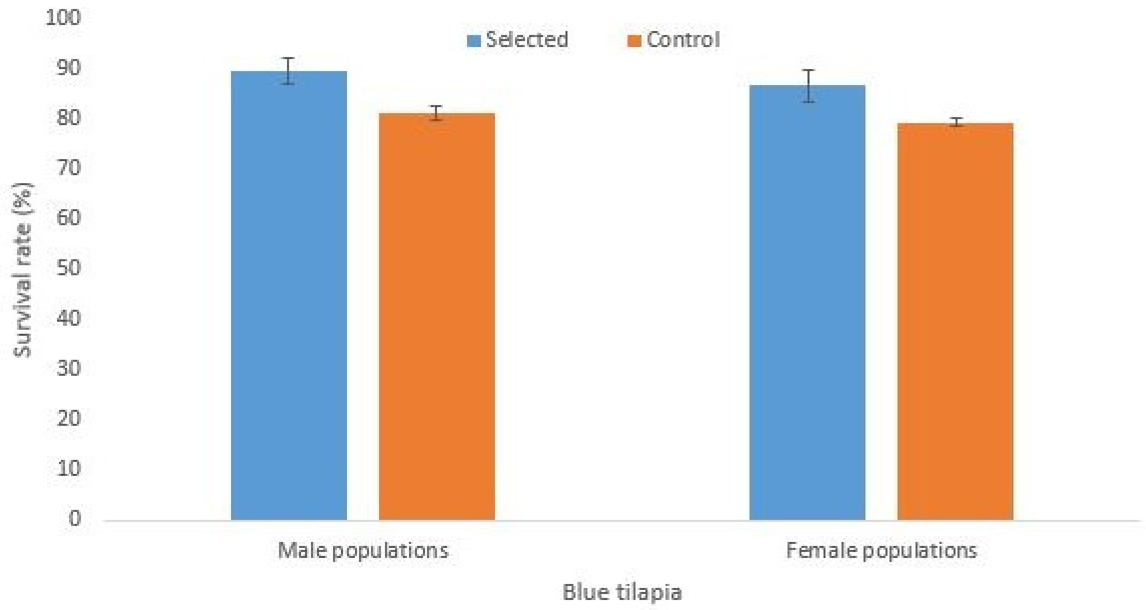

Figure 2. Survival of blue tilapia generation 4 (F-4) male and female population selected and control at brackish water pond

3.2 Heritability and Response to Selection of Blue Tilapia F-4

The heritability value is an important parameter in the program genetic improvement, as it is used as a basis for measuring selection progress. The heritability value in this study is the result of the calculation between the selection response (the difference in the weight of the F-4 blue tilapia selection and the control F-4 weight) divided by the selection differential value of the F-3 blue tilapia. The calculation of the realized heritability value using the F-3 differential selection value of 76.29 g (male) and 63.27 g (female) (Table 2). In this study, heritability analysis was carried out on heritability in the narrow sense of heritability. This analysis was carried out based on the performance of the weights obtained through the results of sampling in ponds. This value was obtained from the results of the evaluation of the population weight of the blue tilapia F-4 which is the fry of the selected F-3 blue tilapia) and the control population of the blue tilapia F-4 (which is the fry of the non-selection F-3 blue tilapia). Ariyanto *et al.* (2014) stated that the power of inheritance of dominant and epistatic genes is not independent of the action of additive genes. So, the calculation of real heritability only considers additive genetic variation without looking at dominant genetic variation and epistasis in the population.

The realized heritability value of the weight character of F-4 blue tilapia reared at 25-30 gL^{-1} salinity was 0.64 in the male population and 0.70 in the female population (Table 2). This value is included in the high category and illustrates that 65%-70% of the weight diversity is influenced by additive genetic diversity, thus giving hope that family selection activities in blue tilapia can increase the average body weight character of the next generation. The high heritability value in this study could be due to the tight limit of individuals selected from each family, which was below 10%. Falconer (1981) and Tave (1995) categorized the h^2 value of quantitative characters in fish with high categories ranging from 0.3 -1.0. The results in this study are similar to the report of Thodesen *et al.* (2013) that the heritability value of body weight of the fourth-generation blue tilapia (G4) selected at low temperatures is 0.40. Then Ninh *et al.* (2014) reported that selected black tilapia reared in ponds with a salinity of 15-20 gL^{-1} had a high body weight heritability value of 0.53%. Likewise, the estimated heritability value of the first-generation black tilapia (F1) from the selection of families kept in ponds is 0.42 (Robisalmi *et al.*, 2019). Meanwhile, Nugroho *et al.* (2017) reported that the heritability value of black tilapia F1 to F-4 as a result of individual selection in freshwater decreased from 0.31-0.25 as a result of the cut-off limit for selected fish was still more than 10%. The high heritability value indicates that the phenotype appearance is due to additive genotype variations and gives hope for a high selection response value, while the low heritability value is due to greater environmental effects than genetic variation (Gjedrem, 2000; Campos *et al.*, 2020).

It is known that the difference in the average weight of the selected F-4 and the control F-4 population is 48.77 g (male population) and 44.33 g (female population) (Table 2). This indicates an increase in the population growth performance of the F-4 blue tilapia by 19.72% and 21.11% from the F-3 population. This value is higher than the report by de Verdal *et al.* (2014) that the selection response of hybrid tilapia (*Oreochromis mossambicus* x *O. niloticus*) fourth-generation (F-4) reared in ponds is 12.5 g or 7.3% per generation. The value of positive and high selection response in this study was determined by the value of the selection differential, as in this study it was known that the value of the selection differential in the previous generation was 53.66 g and 80.79 g and resulting in a high number of selected individuals as broodstock for the formation of the next generation. According to Iswanto *et al.* (2014), the application of a high differential without ignoring the minimum effective population size (Ne) used is expected to produce a positive selection response in the next generation selection program. Although Beaumont *et al.* (2010) stated that a high selection differential cannot be applied if the number of selected individuals produced is only small, because it can increase inbreeding rates through the use of limited broodstock. Factors that influence the selection response include selection differential and heritability of selected characters, sampling error, and environmental influences (Falconer and Mackay, 1996; Gustiano *et al*., 2013). In addition, a higher selection response was also associated with a higher intensity of selection in the male and female parents used compared to the previous generation (Thodesen *et al*., 2012).

Several other research results reported that the accumulated response value of blue tilapia after three generations at a weight of 263 g was 17.7% or 5.5% per generation (Zak *et al*., 2014), while the fourth generation with a weight of 168 g had an accumulated response value selection 14% (Thodesen *et al*., 2013). The success of the GIFT tilapia breeding program was reported by Thodesen *et al.* (2011) that the average selection response of GIFT tilapia generation 1 to generation 6 ranged from 7.4% to 18.7% (mean 11.4%). Then Khaw (2015) reported using a combination family selection method can increase growth with a genetic increase of 10-15% per generation for 10 generations. Likewise, Bentsen *et al.* (2017) stated that the selection response of GIFT tilapia generation 1 to generation 5 ranged from 9-20.1% with an average response per generation of 13.6%. Meanwhile, de Oliveira *et al.* (2016) reported different results where the average value of the black tilapia selection response from the 1st generation to the 5th generation was 4%. The genetic acquisition value of selection activities refers to a high increase in body weight and reflects an important economic aspect. Estimates of genetic gain for body weight vary from 2.3% to 42% per generation, with an overall average of 12.7% (Gjedrem and Rye, 2018). Campos *et al.* (2020) stated that selection activities carried out at the time of enlargement of 6 months compared to 12 months showed the same genetic gain value, which was in the range of 8-31%, it was possible to evaluate and select genetic characters earlier without affecting the results, so that reduce the cost of breeding programs.

4. Conclusion

Selected F-4 blue tilapia had higher growth and survival performance than control F-4 blue tilapia. The realized heritability value of the population growth character of the F-4 blue fish was in the high category of 0.64 (male) and 0.70 (female), with a genetic gain of 19.72% (male) and 21.11% (female).

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Author's Contributions

All authors have contributed to the final manuscript. The contribution of each author is as follows, AR and PS; reared the fish, collected the data, performed growth analysis, drafted the manuscript. BG; devised the main conceptual ideas, analyzed, and evaluated the final data, and done critical revisions of the manuscript. AR; performed designed the figures and tables, finalized the manuscript. All authors discussed the results and contributed to the final manuscript equally.

Conflict of interest

The authors have no conflicts of interest to declare. All co-authors have seen and agreed with the contents of the manuscript and there is no financial interest to report. We certify that the submission is original work and is not under review at any other publication.

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