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RESEARCH PAPER

Revealing Fluctuations in Heterosis with Age in Hybrid Catfish (*Clarias gariepinus*) and Its Utilization at Locations with Different Altitude

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Abstract

This research aimed to evaluate the relationship between the culture periods and the heterosis of hybrid catfish population and its culture in several locations with different altitudes. Induced breeding was conducted to obtain the reciprocal crossing populations of three catfish varieties. Heterosis during nursery and growing up stage was analyzed based on the SGR-length and the SGR-weight, respectively. For multi-locations purpose, the hybrid population with the best heterosis was cultured for 55 days at three different altitudes, that is, <200 m asl, 300-400 m asl, and >400 m asl. The results showed that the highest SGR-length heterosis was found at 68 days of age, about 28.41%, and the highest SGR-weight heterosis was observed at 95 days of age, about 31.28%. This result indicates that the growth of hybrid catfish is better than that of its parents. In the multi-locations test, the highest heterosis of SGR-weight, about 8.39%, was found in the culture location with an altitude of 300-400 m asl. The stability performance analysis showed that the hybrid catfish was categorized as a genotype with dynamic stability. These findings imply that this hybrid catfish can be cultured in various cultivation models and environments with better harvest yield than other populations.

Introduction

Jelaskan Terkait Genetic Incompatibility, Heterosis Fluctuation, Dan GxE

Catfish (*Clarias* sp.) ranks second in the national production of freshwater-cultivated fish in Indonesia after tilapia (*Oreochromis niloticus*). It is reported that around 1.1 million tons of catfish were produced from aquaculture in 2022. However, this amount is not sufficient to meet national catfish needs (MMAF, 2023). To increase catfish production, supply of high-quality fish is needed that can be easily accessed by farmers. This is because the catfish available from farmers are of poor quality so that the productivity is low. Therefore, it is necessary to provide the high-quality catfish. Janssen et al. (2017) and Nugroho (2021) explained that the use of high-quality catfish plays an important role in increasing production. The contribution of superior catfish and the role of technology not only increases production but also increases the efficiency of fish farming (Nugroho et al., 2022).

Improving the quality of fish can be conducted by optimizing the role of dominant variation (*Vd*) and spread across many loci through hybridization (Ariyanto et al., 2022). The success of the hybridization program is measured by a parameter in the form of the hybrid vigor (heterosis), which is the difference between the average of the offspring of the cross (hybrid population) and the average of the parents (Jiang et al., 2023). The heterosis value varies depending on several factors, including the type of fish, the genetic distance of the parental population, the genetic combining ability between populations, and the environmental factors (Ariyanto et al., 2022). Hybridization is a breeding program that relatively quickly produces superior populations compared to selection programs. Therefore, hybridization programs have great potential for application in the aquaculture industry (Rahman et al., 2018). Empirically, several types of hybridized fish have been widely used in aquaculture activities, including tilapia (*Oreochromis niloticus*) (Mohamad et al., 2021), carp (*Cyprinus carpio*) (Wang et al., 2022), catfish (*Pangasius* sp.) (Hatachote et al., 2015), and others.

A hybridization program should be carried out based on the genetic background of each population. The main genetic parameters include genetic variance effect, combining ability, genetic compatibility, and heterotic (Ariyanto et al. 2022). Combining ability in fish breeding is the ability of parents to pass on favorable genes to their offspring during fertilization. It's a key part of genetic improvement and used in breeding programs to develop new lines and hybrids. Genetic compatibility is used in the selection of male and female individuals to be spawned. Genetic incompatibility between males and females will give rise to detrimental traits in the F1 hybrid population. This results in a phenomenon of decreasing fitness in this population. However, the mechanism by which this occurs is not yet known (Šimková et al., 2022), so the effect of positive heterotic or hybrid vigor can still be relied on to obtain adequate seed sources. The heterosis value of a character fluctuates at different ages (Koolboon et al., 2014). Apart from that, the heterosis value in relation to the expression of phenotypic characters in the culture environment is also fluctuating (Chen et al., 2023; Gan et al., 2023). Understanding this will benefit the breeders or farmers in selecting the best hybrid fish population to use in aquaculture. Accurate implementation of the expression of hybrid vigor within a certain maintenance time will increase the margin in aquaculture.

It is known that the phenotypic performance of a cultivated population is influenced by genetics, the environment, and the interaction of the two. In this context, the utilization of hybrid populations is not only influenced by heterosis characteristics but also by the environment. Catfish culture in Indonesia was conducted in many locations with different conditions. The site culture conducted at different altitudes can result in different environmental agroclimatic. These differences are thought to influence the performance of cultured catfish. Apart from that, the adaptation and stability of the catfish culture in different environments also need to be analyzed. This analysis is carried out to determine whether a population has a location-specific genotype or can adapt widely to all locations (Ariyanto et al., 2021). Several pragmatic methods for explaining and interpreting the response of genotypes to environmental variations have been developed. These methods involve statistical analysis to measure the adaptability and stability of genotypes to environmental variations according to different models. One of the models most widely used in general agriculture is the model developed by Eberhart & Russell (1966). This model was developed based on regression of genotype performance against environmental indices.

The research was conducted to evaluate the fluctuations in the heterosis of catfish at different ages and its implication in the culture with different environments. The research results were used to obtain the best hybrid catfish seeds for cultivation under environmental stress conditions.

Materials and Methods

The catfish broodstock used in this study was cultured in the Department of Fisheries and Marine Affairs, Nusa Tenggara Barat Province, Indonesia. The populations originated from African catfish farm in Sukabumi (West Java, identified as Su), Mojokerto (East Java, identified as Mo), and Pandeglang (Banten, identified as Pa). Preliminary study using the Random Amplified Polymorphic DNA (RAPD) analysis with primers of OPA-07, OPA-09, and OPA-11 showed that the Sukabumi (Su), Mojokerto (Mo), and Pandeglang (Pa) populations each had heterozygosity of 0.189, 0.273, and 0.147 and polymorphism levels of 60.0, 70.0, and 40.0, respectively. These results showed that the catfish population from Sukabumi (Su) has the furthest genetic distance from the Mojokerto population (Mo), but is closer to the Pandeglang population (Pa). The broodstocks were maintained in outdoor concrete tanks (2.0x 3.0x0.8 m) which were installed with an aeration system. The water daily temperature was constant at 26.0-30.5°C. Fish were fed commercial pellet feed with a protein content of 40% daily in amounts equivalent to 2% of the total body biomass. The fish were not fed for two days before hormonally treatment for the reproduction induction.

Spawning, Larvae Rearing and Nursery

The broodstocks were crossed reciprocally to produce nine populations, namely three pure lines populations, three hybrid populations, and three reciprocal hybrid populations. Each cross-combination was conducted with four replications. The first step of spawning is filling water in an indoor tarpaulin pond (2.0x1.0x0.6 m) with a depth of 40 cm. The pond is equipped with an aeration system, a heater set at a temperature of 29.0-30.0°C, and a "kakaban" made from palm fiber to attach the eggs. Spawning of the broodstock is stimulated by injecting Ovaprim hormone (Syndel, Canada) at a dose of 0.5 ml per kg of female fish weight, and 0.3 ml per kg of male fish weight. Hormone injection is carried out on the back of the fish by forming an angle of 45° and pointing towards the head. The broodstocks are spawned naturally in this pond, and after five days, the larvae were collected and reared in an aquarium (0.6x0.4x0.4 m) with a water depth of 30 cm. The stocking density of larvae used is 1,000 individuals/aquarium. The larvae were reared for 14 days. In the first week, the larvae were fed *Artemia*, while in the second week, they were fed *Tubifex* worms.

Fish sorting is carried out using a sieve made from a plastic tub with diameter holes of 2 cm. Sorted fish are maintained in aquaria at a density of 1000 fish/aquarium for 14 days. Next, sorting and thinning were carried out at a density of 700 fish/aquarium and kept for 40 days. The feed used at this stage is Tubifex. Sorting and thinning were carried out again with a stocking density of 500 fish/aquarium until the age of 80 days. In this period, the length of individuals from each population is measured every two weeks to estimate the heterosis of growth characteristics related to the ages. During this period, the daily temperature and pH of the rearing media was monitored at 25.0–29°C and 6.2–7.0, respectively.

Grow-out

The grow-out of the populations was carried out using tarpaulin ponds measuring 2.0 x 2.0 x 1.0 m with a water depth of 80 cm. The density used is 90 fish/m^2. During the 55-day growth-out stage, catfish were fed floating pellets with a protein and a fat content of 31 – 32% and 4 – 6%, respectively. The feeding dose is 10% of the biomass with a feeding frequency of 4 times/day (06.00; 12.00; 18.00; and 20.00). Every two weeks, the weight of 75 individuals from each replication was measured to estimate the heterosis of growth characteristics related to the ages. At the end of this stage, all fish, and the amount of feed used in each population was calculated. During the grow-out period, the daily temperature and pH of the water was monitored at 25.0 – 30.0 °C and 6.0 – 6.8, respectively. Minimum and maximum daily temperature fluctuations occur between early morning and the midday.

Multi-locations Test

The study was carried out at the growing stage on hybrid catfish that had the highest heterosis based on the previous test. The two parent populations, the hybrid and the reciprocal hybrid populations, and a catfish population from a local hatchery were used. The fish were cultured in ponds until the age of 135 days at locations with different altitudes, namely the Gurupuk area (<200 m asl), Aik Bukak area (300-400 m asl), and Batu Kumbung area (>500 m asl) (Figure 1). Each treatment was repeated four times. During the multi-location test period, monitoring of the temperature of the culture media was conducted each day. The parameter observed is the specific growth rate of weight (SGR-weight) of each population. Thirty individuals were used as samples for each replication. To evaluate the growth stability of catfish in each location, an environmental index analysis was carried out, which describes the influence of environmental conditions on the phenotypic performance of the catfish.

Parameters and Data Analysis

The study was conducted with completely randomized design research. Data are presented as mean±standard deviation (SD). The parameter was analyzed with analysis of variance (ANOVA), followed by the Duncan's Multiple Range Test (DMRT). The parameters analyzed at the nursery and grow-out stages are specific growth rate (SGR-length and SGR-weight), mid-parent heterosis, and the best-parent heterosis (heterobeltiosis) of the growth rates. The SGR length calculated for fish is 80 days old, and the SGR weight calculated for fish is 135 days old. The mortality rate, survival rate, and feed conversion ratios (FCR) were analysis as supporting data for grow-out stage. In the multi-location test, the parameters observed are the mid-parent heterosis, standard heterosis, and stability performance of specific growth rate based on the body weight (SGR-weight). Mid-parent heterosis was calculated to estimate the hybrid vigor resulting from crossing varieties with their parent. The standard heterosis was calculated to determine the superiority of the hybrid population over the local population used by farmers. Data was analyzed using variance analysis with SPSS 16.0 software. The specific growth rate was calculated as follows:

$$SGR = \frac{\text{Ln } W(L)t - \ln W(L)0}{t} \times 100$$

Where is SGR=specific growth rate; W(L)t=the average weight (W) or length (L) of harvested fish (g); W(L)0=the average weight (W) or length (L) of stocked fish (g); t=the time of rearing fish (day).

The mid-parent heterosis, best-parent heterosis (heterobeltiosis), and standard heterosis were calculated as follows:

$$H_{mp} = \frac{\text{Crosses} - [(AA+BB)/2]}{[(AA+BB)/2]} \times 100\%$$

$$H_{bp} = \frac{\text{Crosses} - P(\text{best parent})}{P(\text{best parent})} \times 100\%$$

$$H_{bp} = \frac{\text{Crosses} - P(\text{best standard})}{P(\text{best standard})} \times 100\%$$

Where is AA=Average body weight or length of sire; BB=Average body weight or length of dam; P=Parent; and P (Standard) is the most catfish variety or population used by local farmers.

The stability performance model developed by Eberhart & Russell (1966) is estimated by carrying out repeated tests in various cultivation environments. This estimation involves environmental index analysis based on the growth rate of the test population at each location. In this estimation, the method for analyzing the stability of a population is to use the average value of the sum of the squares of regression deviations (Eberhart & Russell, 1966; Singh & Chaudhary, 1979), with the linier model as follows:

$$Y_{ij}=\mu+ß_i\ I_j+\delta_{ij}$$

$$I_j=\frac{\sum_I Y_{ij}}{t}=\frac{\sum_i \sum_j Y_{ij}}{tm}$$

Where is Y_{ij} = average of the i[th] population in the j[th] location; μ = average of all populations in all locations; $ß_i$ = regression coefficient of the i[th] population on the environmental index indicating the population's response to the environment; I_j = environmental index, namely the deviation from the population average in a season on all averages; t = number of tested population; m = number of seasons; δ_{ij} = regression deviation of the i[th] population in the j[th] location.

The population is considered stable if the regression coefficient (b_i) is not significantly different from one, and the regression standard deviation (S^2_{di}) is not different from zero based on the student's t-test. The value of b_i and S^2_{di} are approximated by the following formula:

$$ß_i=\frac{\sum Y_{ij}\ I_{ij}}{\sum I_j^2}$$

$$S_{di}^2=\frac{\sum \delta_{ij}^2}{t-2}-\frac{S_e^2}{r}$$

Where is t=number of tested populations; S_e^2=estimation of error variant; r=replication.

The results of the multi-location test will also determine whether the hybrid catfish population was categorized as the specific location population or able to adapt well to varying environments population.

Result and Discussion

Performance and Fluctuations of Heterosis

The length, weight, specific growth rate of length (SGR-length), and weight (SGR-weight) of the nine catfish populations tested were presented in Table 1. As

Figure 1. Site locations of multi-location research for hybrid catfish *Clarias gariepinus*. A: Gurupuk (<200 m asl), B: Aik Bukak (300-400 m asl), and C: Batu Kumbung (>500 m asl).

Table 1. The average standard length, SGR length for 80 days, body weight, and SGR weight for 135 days of the crossing catfish *Clarias gariepinus* populations

Population	Standard length	SGR-length	Weight	SGR-weight
Su × Su	11.9±1.28[ab]	3.75±0.11[a]	123.46±3.26[d]	4.69±0.10[c]
Mo × Mo	12.0±1.27[a]	3.59±0.16[c]	132.19±2.31[c]	4.66±0.06[c]
Pa × Pa	11.6±1.11[b]	3.68±0.06[b]	122.44±3.00[d]	4.64±0.04[c]
Su × Mo	12.3±1.09[a]	3.53±0.10[cd]	156.17±2.01[a]	4.92±0.04[a]
Mo × Su	12.2±1.09[a]	3.57±0.06[c]	152.86±2.27[a]	4.94±0.02[a]
Su × Pa	12.0±1.20[ab]	3.59±0.14[c]	132.75±2.34[c]	4.65±0.15[c]
Pa × Su	12.1±1.10[a]	3.68±0.23[b]	132.16±3.07[c]	4.67±0.05[c]
Mo × Pa	12.0±1.10[ab]	3.66±0.08[b]	137.03±2.75[bc]	4.82±0.13[b]
Pa × Mo	12.0±1.16[ab]	3.48±0.02[d]	143.35±3.41[b]	4.90±0.05[b]

Expl.: The number at the same column followed by different asterisk shows the significant difference, Su=Sukabumi strain, Mo=Mojokerto strain, Pa=Pandeglang strain.

supporting data, mortality rate, survival rate, and feed conversion ratios (FCR) during the grow-out stage were presented in Table 2. In general, there were significant differences in the standard length of each cross at 80 days of age (P<0.05). The highest standard length was obtained in the Sukabumi×Mojokerto (Su×Mo) catfish cross, while the lowest standard length was obtained in the Pandeglang population. Similar results were also obtained on the body weight performance of catfish at 135 days of age. The highest weight was obtained from the Su×Mo cross, while the Pandeglang population produced the lowest weight. SGR measured in fish up to 80 days old and from 80-135 days old were significantly different (P<0.05), varied from 3.48% to 3.75%/day and from 4.64 to 4.94%/day, respectively (Table 1). Table 2 showed that the mortality rate of all populations in this study was less than 10%, resulting in a high survival rate. The level of FCR was relatively low, ranging from 1.14 to 1.20.

Estimates of heterosis and heterobeltiosis SGR-length of crossed catfish up to 80 days of age and SGR-weight up to 135 days of age are presented in Table 3 and Table 4. Based on Table 3, the heterosis of SGR length fluctuates at different ages. The highest heterosis was achieved when reared until 68 days of age and the lowest at 80 days of age. In general, the average heterosis of crossed catfish up to 80 days of age is negative, meaning that the average SGR length of the hybrid and the reciprocal population is lower than that of the two parents. Similar results also occurred in heterobeltiosis estimation, which showed that none of the average SGR-length of the hybrid population was better than that of the best parent population.

Based on Table 4, it can be seen that SGR-weight heterosis also fluctuates at different ages. The highest heterosis was achieved when reared until 95 days of age and the lowest at 125 days old. In general, although the heterobeltiosis value is negative, the hybrid population between catfish from Sukabumi and Mojokerto (Su×Mo) has a heterosis of 6.07, which indicates that this hybrid population has good potential for cultivation by farmers. Based on this result, the hybrid population Su×Mo was cultured in a multi-location test with different altitudes.

Table 2. Mortality rate, number of feed, and feed conversion ratios (FCR) of the crossing catfish *Clarias gariepinus* population at growth-out stage

Population	Mortality rate (%)	Survival Rate (%)	Number of Feed (kg)	FCR
Su × Su	6.43	93.57	180	1.20
Mo × Mo	7.50	92.50	185	1.18
Pa × Pa	9.86	90.14	170	1.19
Su × Mo	2.36	97.64	222	1.14
Mo × Su	5.00	95.00	215	1.14
Su x Pa	8.00	92.00	183	1.17
Pa × Su	8.93	91.07	180	1.17
Mo × Pa	7.14	92.86	190	1.16
Pa × Mo	9.79	90.21	195	1.16

Table 3. SGR-length heterosis and heterobeltiosis according to 26-80 days age of the crossed catfish *Clarias gariepinus*

Age (day)	Heterosis				Heterobeltiosis			
	$H_{Su×Mo}$ (%)	$H_{Su×Pa}$ (%)	$H_{Mo×Pa}$ (%)	Average	$H_{Su×Mo}$ (%)	$H_{Su×Pa}$ (%)	$H_{Mo×Pa}$ (%)	Average
26	-1.73	-1.79	-2.86	-2.12	-3.19	-0.49	-3.02	-2.23
40	-7.07	-5.30	-1.91	-4.78	-10.09	-7.40	-7.28	-8.26
54	-5.80	1.15	-3.94	-2.85	-6.43	1.15	-4.59	-3.29
68	32.64	21.12	31.55	28.41	26.56	13.65	17.42	19.21
80	-75.25	-49.33	-65.32	-63.57	-73.48	-48.63	-62.36	-61.49
26-80	-4.52	-2.84	-3.24	-3.53	-6.19	-3.34	-5.44	-4.99

Expl.: Su = Sukabumi strain, Mo = Mojokerto strain, Pa = Pandeglang strain.

Table 4. SGR-weight heterosis and heterobeltiosis according to 95-135 days age of the crossed catfish *Clarias gariepinus*

Age (day)	Heterosis				Heterobeltiosis			
	$H_{Su×Mo}$ (%)	$H_{Su×Pa}$ (%)	$H_{Mo×Pa}$ (%)	Average	$H_{Su×Mo}$ (%)	$H_{Su×Pa}$ (%)	$H_{Mo×Pa}$ (%)	Average
95	49.99	12.76	32.22	31.92	-32.38	-14.49	-14.49	-22.81
110	-28.27	-0.59	-7.50	-11.86	-44.60	-21.47	-21.47	-34.40
125	-29.43	-4.18	-34.89	-23.43	-35.04	-5.64	-5.64	-17.40
135	-21.12	-2.60	-19.91	-14.69	86.92	34.43	34.43	53.57
95-135	6.07	0.01	4.07	3.38	-6.19	-3.34	-5.44	-4.99

Expl.: Su = Sukabumi strain, Mo = Mojokerto strain, Pa = Pandeglang strain.

Utilization of Hybrids in Different Altitude Locations

The SGR weight of five catfish populations cultured at different altitudes is presented in Table 5, and the variance analysis of them is presented in Table 6. In general, there are no significant differences in SGR weight among all populations (Table 5). However, it can be seen that the Su × Mo hybrid population has relatively faster growth than the other populations, while the local catfish population is the slowest. This research noted that the survival rate of all populations in multi-location test was 100%. Based on the analysis of variance (Table 6), there are no significant effects of the population (genotype), altitudes (environment), and interaction of the two to the SGR weight. However, the influence of the population is the highest compared to the altitude of the location and the interaction of these two factors.

Analysis of heterosis for SGR-weight of the hybrid catfish cultured in three different altitudes was served in Table 7. The highest mid-parent heterosis based on SGR weight was found in catfish cultured at an altitude of 300-400 m asl. The heterosis decreases at culture location with altitudes of <200 m asl and >500 m asl. The phenomenon of decreasing heterosis with increasing age occurs at all altitude levels of the site. The highest heterosis value occurs at the age of 110 days at an altitude of 300-400 m asl and the age of 95 days for locations above 500 m asl.

Table 6 also showed that the standard heterosis for hybrid catfish against local catfish is 9.93-12.09%. This result shows that the hybrid catfish population has a better growth rate than the local population. Slightly different from mid-parent heterosis, the standard heterosis for catfish in this study was getting higher with increasing altitude of the culture location. This result indicates that the local catfish are thought to be less able to adapt well to the high altitude of the cultivation location.

Stability of the Growth Rate

Analysis of daily water temperature in three locations at different altitudes during the study was presented in Figure 2. The daily temperature in each pond fluctuated during the cultivation period. The average daily temperature at an altitude of <200 m asl and between 300-400 m asl is relatively not significantly different. However, the daily temperature of locations with an altitude of more than 500 m asl is significantly lower than other locations.

An environmental index analysis referring to the SGR-weight of the catfish population was presented in Table 8. The analysis of the environmental index showed

Table 5. SGR-weight of five populations of catfish *Clarias gariepinus* cultured in three different levels of altitudes (m asl)

Populasi	<200	300-400	>500	Means
Mo × Su	4.43±2.45	4.51±2.37	4.42±2.40	4.46±0.05
Su × Mo	4.62±2.48	4.60±2.51	4.60±2.37	4.61±0.02
Mo × Mo	4.30±2.42	4.24±2.48	4.21±2.18	4.25±0.04
Su × Su	4.24±2.17	4.22±2.32	4.22±1.88	4.23±0.01
Lo × Lo	4.12±1.99	4.11±2.11	4.09±1.60	4.11±0.02
Means	4.43±0.19	4.34±0.21	4.31±0.20	4.33±0.20

Note: Su=Sukabumi strain; Mo=Mojokerto strain, Lo=Local strain, m asl=meters above sea level

Table 6. Analysis of variance of SGR-weight of five populations of catfish *Clarias gariepinus* cultured in three different levels of altitudes (m asl)

Source of Variation	SS	df	MS	F	P-value	F crit.
Altitudes (E)	0.016579	2	0.008289	0.001618	0.998383	3.204317
Populations (G)	1.923178	4	0.480795	0.093851	0.983919	2.578739
Interaction (G×E)	0.024714	8	0.003089	0.000603	1	2.152133
Within (Error)	230.5331	45	5.122958			
Total	232.4976	59				

Table 7. The mid parents heterosis and standard heterosis of hybrid catfish *Clarias gariepinus* cultured at different levels of altitudes (m asl)

Ages (day)	Mid parents Heterosis			Standard heterosis		
	<200	300-400	>500	<200	300-400	>500
135	-0.52	10.55	4.61	9.79	18.15	10.64
125	5.73	-0.30	1.06	-1.10	-2.06	-1.02
110	11.99	19.58	4.17	4.87	7.68	-5.10
95	6.21	3.82	11.83	26.16	20.70	43.84
Averages	5.85	8.39	5.42	9.93	11.12	12.09

that cultivation at an altitude of <200 m asl and between 300-400 m asl has the same value, whereas cultivation locations with an altitude of >500 m asl have a lower environmental index. These results indicate that the environment above 500 m asl has conditions that tend to be unfavorable for the growth of catfish, especially related to lower water temperatures (Figure 2).

The results of the stability analysis referring to the $ß_i$ and Sd_i^2 were presented in Table 9. According to Eberhart & Russell (1969), the population is considered to be stable if the population's $ßi$ is not significantly different from one, and Sd_i^2 is not significantly different from zero. The stability analysis of the growth rate of the catfish at three different cultivation sites is presented in Table 8. The analysis showed that the regression coefficient ($ß_i$) for catfish is not significantly different from one, but the regression deviation (Sd_i^2) is not equal to zero. This result showed that the catfish population tested in this study has a dynamic growth rate. In suitable locations, the five catfish populations tend to have better growth rates. However, the regression deviation (Sd_i^2) in the hybrid catfish population is greater, indicating that this population has better adaptability than other populations, especially the local catfish population. The local catfish have the smallest regression deviation (Sd_i^2), indicating that the population has a relatively constant growth rate.

Discussion

The phenotypic performance of catfish culture in this study was quite good, indicated by an average SGR-length of more than 3.5%/day and an average SGR-weight of more than 4.5%/day. This value is much better than the study conducted by Primashita et al. (2017) on catfish (*Clarias gariepinus*) raised in an aquaponic system that had an SGR-weight of 0.019-0.025%/day. The performance of the catfish in this study was also better than the study reported by Arief et al. (2014) on Sangkuriang catfish (*C. gariepinus*) with an SGR-weight

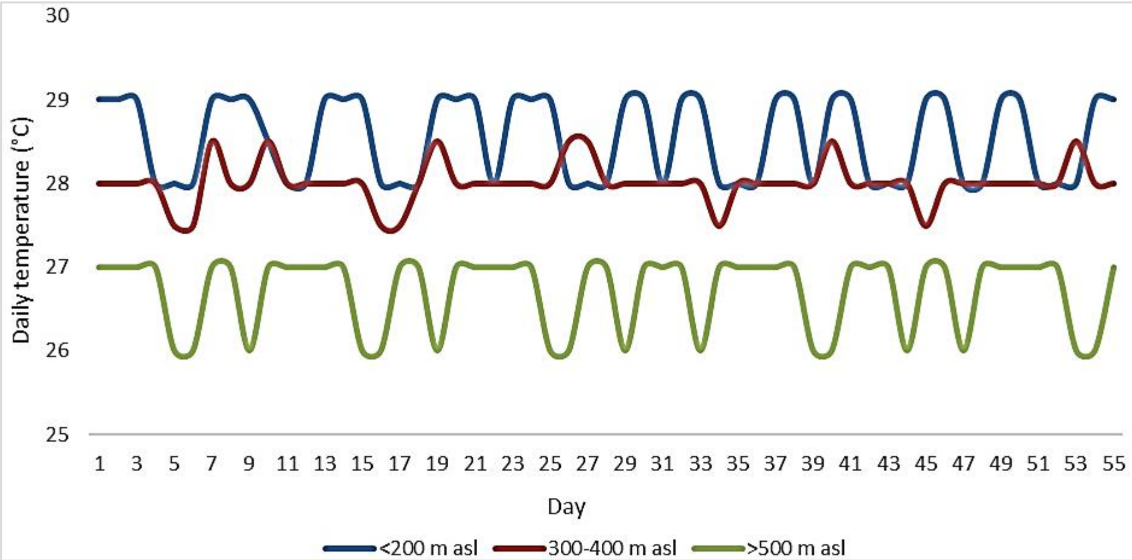

Figure 2. The fluctuation of daily temperature in three locations of catfish *Clarias gariepinus* culture for 55 days.

Table 8. Environmental indexes of each altitude for catfish *Clarias gariepinus* culture based on the SGR weight

Altitudes (m asl)	Environmental indexes
<200	0.01
300-400	0.01
>500	-0.02

Table 9. The values of $ß_i$ dan Sd_i^2 of the five populations of catfish *Clarias gariepinus* which cultured in three different levels of altitudes for 90 days.

Populations	$ß_i$	Sd_i^2
Mo × Su	1.07^{ns}	10.86
Su × Mo	1.09^{ns}	11.27
Mo × Mo	1.05^{ns}	10.73
Su × Su	0.95^{ns}	7.72
Lo × Lo	0.84^{ns}	5.19

Note: Su=Sukabumi strain; Mo=Mojokerto strain, Lo=Local strain, ns=not significantly different from 1.

of 1.73-2.88%/day. The high growth rate of the catfish in this study has an impact on the efficiency of feed use so that the FCR level at the end of the study was low, which was less than 1.20. The use of pellet feed with a crude protein content of between 31-33% is suspected to be one of the causes of good performance of fish in this study. According to Langi et al. (2024) the minimum protein requirement in catfish rearing feed is 28-32%. In addition to the high growth rate, the mortality rate at grow-out stage of catfish in this study was also relatively low, resulting in high survival rate in all cross populations. Even in multi-location tests, the survival rate of all test fish was 100%. The adequacy of protein requirements in feed in this study is thought to play a role in reducing cannibalism in catfish. Good performance of growth, survival, and FCR are the main keys to successful catfish culture. These parameters have a direct impact on catfish production at harvest time.

Heterosis plays an important role in the success of a hybridization program. It was explained by Tave (1995) that the success of hybridization, especially as indicated by heterosis, is influenced by several factors, such as the age of fish, genetic distance, and combining ability between parental genotypes, and the environment. Different types of fish will produce different heterosis effects. Guy et al. (2009) reported that hybrid silver perch populations reared at different ages would produce different heterosis values. The irregular growth rate between fish age periods in each population is thought to cause differences in growth rates between the hybrid population and its two parents. In this study, the growth rate of hybrid catfish was faster than its parents, resulting in heterosis which increased with age. These results are increasingly visible at the rearing stage, where the hybrid population has a significantly faster growth rate than the average of its two parents. Even in the 125-135 days-old period, hybrid catfish heterobeltiosis was 53.57%, which means that the hybrid population had a growth rate of 53.57% better than the growth rate of its best parents. Even though the final average heterobeltiosis value was negative, the average heterosis value of the hybrid catfish population was still positive, ranging from 0.01% in the Sukabumi × Pandeglang population cross to 6.07 in the Sukabumi × Mojokerto population cross. The instability of hybrid vigor in body weight parameters also puts pressure on farmers in their business. Operational cost efficiency becomes more difficult when compared to the hybrid vigor effect that can be controlled in fish farming. Considering the freshwater fish business which is very vulnerable to price fluctuations in the market, efforts to produce superior fish need to be carried out in an integrated manner with the cultivation technology used.

Another factor that influences heterosis analysis is the different genetic distances between the two parental populations. One of the easiest estimates of the genetic distance between two or more fish populations is based on the location of origin of the populations. Several studies related to the genetic distance of fish show this phenomenon, such as in tilapia, *O. niloticus* (Tibihika et al., 2020), common carp, *Cyprinus carpio* (Lalramnunsanga et al., 2024), gourami, *Osphronemus gouramy* (Nugroho et al., 2019) and catfish, *C. gariepinus* (Alal et al., 2021). However, the distance from the population's origin does not always result in a large genetic distance. This occurs in populations developed from the same genotype but carried out in different places. This usually happens to commodities that are largely used in society so distribution between regions is high (Ye et al., 2022). Apart from long genetic distances, gene compatibility or combining ability (general and specific combining ability) between genotypes also plays an important role in the success of hybridization. Even though there is a large genetic distance, if the two genotypes do not have a large combining ability it will not produce a good hybrid population. It is even possible that there will be a failure in the hybridization process (Odin et al., 2024). In this study, the parent populations used came from three different locations, namely Mojokerto (East Java), Sukabumi (West Java), and Pandeglang (Banten). The highest heterosis value was obtained in the Sukabumi × Mojokerto, followed by Mojokerto × Pandeglang and the lowest was the Sukabumi × Pandeglang. These results are in accordance with preliminary study which stated that the genetic distance between the Mojokerto population and the Sukabumi and Pandeglang populations is further than the population from Sukabumi to the Pandeglang population. These results follow the distance from the origin of these parent populations, where geographically the Mojokerto region is a location that is quite far from Sukabumi and Pandeglang. The distance between Sukabumi and Pandeglang is relatively close. Apart from geographical distance, the catfish population developed in Mojokerto is the Masamo catfish (Alviani, 2017), while the populations developed in Sukabumi and Pandeglang are thought to originate from the Sangkuriang catfish (Feriyanto, 2019). These two types of catfish are widely developed in NTB, where this research activity was carried out (Nugroho & Putera, 2018). Masamo catfish was developed by a private company in Mojokerto, while Sangukriang catfish was created by the Center for Freshwater Cultivation Development in Sukabumi. DNA analysis carried out by Nugroho & Putera (2018) shows that the genetic distance between Masamo and Sangkuriang catfish is relatively far, so they have the potential to produce a good hybrid population if crossed.

Besides genetic factors, different environments will also influence the phenotypic performance of the population being reared. Over there, Wang et al. (2022) explained that different culture environment influences the heterosis produced in hybridization programs of fish. Each of these different populations has a different adaptability in responding to environmental conditions. These different responses will indirectly affect the heterosis value obtained. In this study, the altitude of different locations affects the ambient temperature in that environment. The higher altitude has the lower water temperature. This condition is thought to influence the performance of the catfish, which was cultured in each location, especially at the altitude more than 500 m asl. The effect of lower temperatures on reducing the growth rate of several types of catfish has been reported by Singh et al. (2009), Suja et al. (2009), and Orina et al. (2016). Lower temperatures when rearing catfish cause an increase in the utilization of the body's energy sources, especially those originating from fat and protein to maintain the body's metabolism. This has an impact on the phenotypic performance of farmed fish, such as slower growth rates, and lower resistance to disease and environmental changes, thus potentially resulting in higher mortality rates.

Fish are poikilothermal and therefore changes in water temperature will affect growth, feeding, metabolism, and other biological processes. Chen et al. (2023) reported that when the water temperature falls below the suitable range, fish will show a decline in feeding capacity, weakened swimming vitality, and low rates of survival. As explained by Bowyer et al. (2013), under optimal temperatures, food energy partitioned into fish growth can be maximized. The average daily temperature in this study ranged from 26-27 at locations above 500 m asl, 27.5-28.5 at locations with an altitude of between 300-400 m asl, and 28-29 at locations less than 200 m asl. In general, temperature variations in the three locations are still within the tolerance threshold for catfish. So even though there are differences in average temperature at each location, the decrease in catfish performance is relatively not significant. However, the results of this research are a reminder for farmers that lower environmental temperatures will cause the growth rate of catfish to become slower.

The concept of stability of a population according to Eberhart & Russell (1966) is the relative comparison between populations in a set of tests under various environmental conditions. The stability of the populations tested is based on the regression response of the population's growth to its environmental index. Based on this concept, the adaptability and stability of a population is determined by the value of the regression coefficient and the regression deviation. Stability analysis in this study indicates that the hybrid catfish have a good response to changes in environmental conditions. This good response shows that hybrid catfish have broad adaptability to varying environmental conditions. In the context of fish culture, genotypes are divided into two categories, namely location-specific genotype and broad adaptability genotype. Location-specific genotypes have maximum phenotypic performance in a good environment and suit their needs, while genotypes with broad adaptability have relatively stable phenotypic performance in all cultivation locations. This genotype is suitable for anticipating possible harvested failures caused by sudden changes in environmental conditions. Populations with broad adaptability will have stable harvest yields (De Vita et al., 2010). It was further explained by Mohammadi et al. (2010) that a population that has adaptability with the same productivity in various cultivation environments shows static stability, while adaptability that follows environmental indices shows dynamic stability. Based on this analysis, this hybrid catfish can be categorized as a genotype with dynamic stability in different environmental conditions.

Providing genotypes that are able to adapt widely to different environmental conditions is the key to successful fish culture with different conditions such as in Indonesia (Ariyanto et al., 2021). In addition, this genotype is also a mainstay in dealing with climate change that occurs globally, which has an impact on the aquaculture production (Maulu et al., 2021), because it has good plasticity capabilities in dealing with changing environmental conditions. In addition to environmental conditions, the different abilities of farmers in providing various systems and technologies also require the provision of genotypes that can be cultivated with various cultivation models. The hybrid catfish in this study is suitable for use in aquaculture activities with various cultivation systems with diverse agroclimatic conditions.

Conclusion

Heterosis of the hybrid catfish was influenced by a combination of its parents, age, and culture environment. The best hybrid catfish population in this research was obtained from a cross between Sukabumi (Su) females and Mojokerto (Mo) males, with a heterosis for growth rate of 6.07%. This hybrid population has the best phenotypic performance in all environmental conditions at different altitudes. Based on the stability analysis, this hybrid population was categorized as the genotype that has dynamic stability, which will produce high yields under the right environmental conditions and cultivation technology.

Ethical Statement

This study has complied with the guidelines of animal ethical treatment for the management and use of experimental animals. There is no specific treatment that can harm the fish. All fish are cultivated with optimal feed and water conditions. This article also does not require IRB/IACUC approval because there are no human participants.

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Author Contribution

First Author: Conceptualization, Data analysis, Writing -original draft, Writing -review and editing; Second Author: Data curation, Data analysis, Writing -original draft; Third Author: Data curation, Data analysis; Fourth Author: Conseptualization, Investigation, Supervision, Resources, Methodology, Writing original draft, Writing -review and editing.

Conflict of Interest

The authors declare that we have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

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