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HYBRID VIGOR AND GROWTH PERFORMANCE OF HYBRID MAHSEER (*Tor* spp.) IN GROW-OUT

Yogi Himawan¹, Imron¹, Otong Zenal Arifin¹, Jojo Subagja¹, Supriyanto¹, and Joni Haryadi¹

⁹National Research and Innovation Agency, Research Center for Fisheries

Jl. Raya Bogor KM.47, Bogor, West Java, Indonesia

"National Research and Innovation Agency, Research Center of Applied Zoology

JI. Raya Bogor KM.47, Bogor, West Java, Indonesia

"National Research and Innovation Agency, Research Center of Appropriate Technology

JI. KS Tubun No 5, Subang, West Java, Indonesia

"Research Institute for Fish Breeding, Ministry of Marine Affairs and Fisheries Republic of Indonesia

JI. Raya Pantura No.2-Sukamandi, Subang, West Java, Indonesia

(Received: November 11, 2024; Final revision: May 20, 2025; Accepted: May 20, 2025)

ABSTRACT

Mahseer (Tor spp.) is a freshwater fish commodity with a high market value. However, its slow growth to reach consumable size has resulted in inadequate production. The aim of this study is to analyze the hybrid vigor - that are heterosis, heterobeltiosis, and growth performance - of a crossbred population of Mahseer (Tor spp) resulting from interspecies breeding. Broodstock of Mahseer from three different species were prepared for reciprocal breeding, producing nine offspring populations consisting of six hybrid offspring and three purebred offspring populations. The breeding process was carried out using artificial breeding methods, employing intramuscularly injected breeding stimulant hormones. Results show that the hybrid population of Tor soro and Tor tambroides exhibits better heterosis and heterobeltiosis in final weight, specific growth rate, and survival rate. The crossbreeding of Tor soro and Tor tambroides also exhibited better growth performance compared to other crossbred population in terms of final weight growth, specific growth rate, and survival rates, which were 64.20 g, 1.42%/day, and 100%, respectively.

KEYWORDS: Final growth; SGR; survival rate; interspecies breeding

INTRODUCTION

Mahseer (*Tor* spp.) is a indigenous fish found in several regions in Indonesia (Rizkiya *et al.*, 2024), such as Sumatra, Java, and Kalimantan. There are four known species of Mahseer fish in Indonesia, namely *T. tambroides, T. douronensis, T. tambra, and T. soro* (Kottelat *et al.*, 1993; Roberts, 1999). Mahseer fish can now be successfully spawned and developed through induced breeding techniques and the species has also adapted to artificial feed. Ingram *et al.* (2005) reported successful captive spawning and rearing of mahseer, laying a foundation for future research aimed at enhancing production.

The presence of various Mahseer species in Indonesia offers valuable genetic diversity that can be uti-

E-mail: yogihimawan@yahoo.com

lized in the creation of superior fast growing Mahseer fish through hybridization. Hybridization is a process of crossbreeding between genetically distinct individuals - either within the same species (intraspecific) or between species (interspecific)- with the aim of producing offspring with superior performance. This method is used in breeding between species, genera, subfamilies, and even families of aquatic animals. The main goal is to obtain offspring with better performance than both parental species in terms of survival rates, growth rates, flesh quality, fertility, and stress resistance. The difference between the average offspring of the crossbreeding and the average performance of the individuals forming it allows the creation of offspring with better performance. Falconer & Mackay (1996) explained that through hvbridization, the proportion of heterozygous genes can be increased that potentially increasing performance traits, while the proportion of homozygous genes tends to decrease. Research indicates that

[#] Correspondence: National Research and Innovation Agency, Research Center for Fisheries

hybridization in fish can produce seeds with specific advantages, such as faster growth, consistency in quality, increased survival and stress tolerance, controlled reproduction by creating sterile fish, and improved feed conversion efficiency (Kusmardani *et al.*, 2021).

Hybridization utilizes the property of hybrid vigor or heterosis, which is the difference between the average of the offspring's crossing results and the average of their parents. According to Agustiani et al. (2019), the development or breeding of hybrid is based on the phenomenon of heterosis and heterobeltiosis, which is a condition where the filial (F1) hybrid will perform better compared to one or both of its parents. Heterosis is defined as the performance of the offspring that exceeds the average performance of its two parents, while heterobeltiosis refers to the performance of the F1 hybrid that surpasses the performance of the best parent (Crow, 1999). The value of heterosis and heterobeltiosis indicates the success of the hybridization program. Several studies on hybrid heterosis have been conducted on various fish species (Ariyanto & Listiyowati, 2011; Nugroho et al., 2015; Radona et al., 2015). Positive heterosis values indicate an improvement in offspring performance compared to their parents, while negative heterosis values indicate a decrease in hybrid performance compared to the parents' average (Warwick et al., 1995). Heterosis values explain or describe a condition of comparison between the average of the offspring and the average of their two parents, particularly determining whether a crossbreeding will result in offspring with better or worse characteristics compared to their parents. Several studies indicate that hybridization in fish can result in high heterosis, yielding offspring with specific advantages such as faster growth and uniformity.

Hybridization has become an effective method to obtain desired characteristics or improve the quality of cultured fish. Previous studies show that better growth rates were achieved in many fish species, such as tilapia, salmonids, carp, oysters, catfish, and abalone through interspecific hybridization (Al-Harbi, 2016; Botwright, 2015). Crosses between different species also resulted in improved environmental and disease tolerances. However, some hybrid offspring showed lower or equal growth rates compared to their parents (Cao et al., 2016), and some hybridization produced sterile offspring or only fertile offspring of a single gender. Research on hybrid grouper fish showed that their growth rate was twice that of commonly cultured leopard grouper (Chaniago, 2020). In aquaculture, the purpose of hybridization is to obtain fish that have advantages over both parents or a combination of their respective advantages. The aim of this study is to analyze the values of heterosis, heterobeltiosis, and the growth of hybrid Mahseer fish at the grow-out stage. Hybrid Mahseer fish for fast growth characteristics needs to be conducted to support the national-scale aquaculture industry. The presence of superior fast growing hybrid Mahseer fish is expected to increase the interest of breeders and shorten the culture period while maintaining consistently high harvested biomass.

MATERIALS AND METHODS

Seed preparation

The Mahseer broodstock from three species are prepared for reciprocal breeding, resulting in three purebred and six hybrid strains consisting of six crossbreed strains and three purebred strains. The breeding method involves artificial propagation with the assistance of breeding-stimulating hormones injected intramuscularly (Eman et al., 2020). The test container uses ponds with concrete walls and bottom measuring 5 m x 5 m x 1 m, with two replications. The test material consists of nine population of Mahseer fish seeds, namely ST (*Tor soro* ⁰/₊ x *T. tambroides* [∧]), TS (T. tambroides $\stackrel{\circ}{_{+}}$ x T. soro $\stackrel{\circ}{_{-}}$), SD (T. soro $\stackrel{\circ}{_{+}}$ x T. douronensis \mathcal{B}), DS (*T. douronensis* $\stackrel{Q}{+}$ x *T. soro* \mathcal{B}), TD (T. tambroides $\stackrel{0}{+}$ x T. douronensis $\stackrel{1}{\triangleleft}$), DT (T. douronensis $\stackrel{\circ}{\downarrow}$ x T. tambroides $\stackrel{\circ}{\lhd}$), SS (T. soro $\stackrel{\circ}{+}$ x T. soro ♂), TT (T. tambroides x T. tambroides), DD (*T. douronensis* $\stackrel{0}{\downarrow}$ x *T. douronensis* $\stackrel{1}{\triangleleft}$). The initial weight and body length of Mahseer seeds from all strains are 5.63 \pm 2.19 g and 6.05 \pm 1.07 cm respectively

Data collection

Testing was conducted with a stocking density of 30 fish/ponds for a duration of 3 months. Feeding was done twice a day with commercial pellets containing 30-32% protein, with the ration of to 7.5 to 5% fish body weight in the 1st and 2nd months and 2.5% in the 3rd month. The pellet size was adjusted based on the gradual mouth opening size of Mahseer fish in the first, second, and third months. Sampling of fish weight for calculating the amount of feed provided was carried out every month. In this study, sampling was carried out by selecting 10% of Mahseer fish from each population through random sampling methods to ensure representativeness. The sampled fish were then placed in the aerated containers with secure lids to maintain optimal oxygen levels and reduce handling stress. To accurately assess growth parameters, each Mahseer fish was anesthetized in a phenoxyethanol solution prepared at a concentration

of 200 mg/L to ensure gentle sedation without harming the fish. Once the fish reached a fully anesthetized state, they were carefully weighed using a highprecision digital scale to obtain growth performance data. After weighing, the fish were promptly transferred to aerated containers to facilitate recovery, ensuring they remained in well-oxygenated conditions until they regained full consciousness. In field measurements of water quality, including temperature, dissolved oxygen, pH, and geographical information around the test pond, were conducted to understand the maintenance medium conditions to support the data collected. The value of heterosis is calculated using a formula:

$$H(\%) = \frac{F1 - [\frac{P1 + P2}{2}]}{[\frac{P1 + P2}{2}]} \times 100$$

Note :

H = Heterosis value (%)

F1 = Cross offspring

P1, P2 = Offspring of parental populations

Heterobeltiosis

Heterobeltiosis (high-parent heterosis) was determined as:

Heterobeltiosis (%) =
$$\frac{F1 \text{ hybrid} - BP}{BP} x100$$

Note:

- F1 = Mean value of F1 hybrids
- BP = F1 best parent (the highest performance of the parents)

Specific growth rate

The specific growth rate calculated using the formula:

$$SGR = (Ln Wt-Ln W_0)/t \times 100\%$$

Note :

SGR = Specific growth rate (%/day)

Wt = Average of final weight

 W_0 = Average of initial weight

t = Cultured time (days)

Survival rate

To calculate the survival rate (SR), the formula is:

$$SR = [Nt]/N_0 \times 100\%$$

Note :

SR = Survival rate (%)

Nt = Amount of final individuals

 N_0 = Amount of initial individuls

The data analysis of the research results utilizes one-way ANOVA to determine differences among treatments.

RESULTS AND DISCUSSION

Heterosis and heterobeltiosis for growth

Final body weight

The analysis of final body weight characteristics reveals that hybridization has a significant impact on growth performance (p < 0,05). Among the different hybrid combinations, the crossbreed between *T. soro* x *T. tambroides* exhibit the highest final body weight compared to other populations (Figure 1).



Figure 1. The final body weight of 9 crosses (average \pm standar deviation) (p<0.05).

Note : ST (Tor soro ♀ x T. tambroides ♂), TS (T. tambroides ♀ x T. soro ♂), SD (T. soro ♀ x T. douronensis ♂), DS (T. douronensis ♀ x T. soro ♂), TD (T. tambroides ♀ x T. douronensis ♂), DT (T. douronensis ♀ x T. tambroides ♂). The highest average final body weight achieved in the crossbred population of ST 64.20 g, followed by the TS, TD, DS, SD, DT, DD, SS, and TT as 48.10 g, 47.10 g, 46.50 g, 41.00 g, 37.60 g, 27.95 g, 24.15 g, and 22.50 g respectively. In general, the growth performance of the ST crossbreeding population is significantly different (p<0.05) compared to other population. The ST crossbreeding population is considered to express the growth advantage genes genetic material better than other populations during the grow-out phase in the pond.

Specific growth rate

The specific growth rate of the hybrid Mahseer fish population during the grow-out stage showed different performances. In this research, the cross of Mahseer fish did not significantly affect (p > 0.05) specific growth rate (Figure 2).

The highest specific growth rate was achieved in the ST crossbreed, reaching 1.42%/day, followed by the DD, TD, DS, SS, DT, SD, TS, and TT as 1.04%/day, 0.95%/day, 0.95%/day, 0.92%/day, 0.86%/day, 0.84%/day, 0.75%/day, and 0.72%/day, respectively. Overall, the ST crossbreed population had highest on the specific growth rate compared to other populations.

Growth is a desired trait for genetic improvement in aquaculture. Faster growth can be caused by dominant variations (Tave, 1993) or an increase in the number of polymorphic loci in individuals. Moreover, faster growth is also due to increased heterozygosity associated with growth improvement and other traits related to fitness that can affect characteristics such as growth stability, feed conversion efficiency, and oxygen metabolism. Research on interspecies hybridization between African catfish (*Clarias gariepinus*) and Vundu catfish (*Heterobranchus longifilis*) in in-



Figure 2. Specific growth rate of 9 crosses (average \pm standar deviation) (p < 0.05).

Note : ST (Tor soro ♀ x T. tambroides ♂), TS (T. tambroides ♀ x T. soro ♂), SD (T. soro ♀ x T. douronensis ♂), DS (T. douronensis ♀ x T. soro ♂), TD (T. tambroides ♀ x T. douronensis ♂), DT (T. douronensis ♀ x T. tambroides ♂).

tensive concrete tanks reported that the hybrid population grew faster compared to their purebred strains. Improved growth and other phenotypic traits were also found in the crosses between common carp (*Cyprinus carpio*) and rohu carp (*Labeo rohita*), mrigal carp (*Cirrhinus mrigala*), and catla carp (*Catla catla*). Falconer & Mackay (1996) revealed that hybridization can lead to an increase in the number of heterozygous genes and can reduce the number of homozygous genes.

The results indicated that crossbreeding involving *T. soro* parents, both male and female, significantly impacted the length growth of the produced fish. Conversely, when crossbreeding involved parents from the *T. douronensis* species, both male and female, the weight growth characteristics of the fish produced were higher. Several studies have indicated that through hybridization in fish, offspring with specific advantages such as faster growth and better consistency can be produced. According to Wang et al. (2024), hybrid catfish such as the cross between channel catfish and blue catfish, have superior growth rates that dominate over half of the total catfish production in the United States. It also in line with research of hybridization of Suminoe oysters that revealed better performance of the hybrids in both northern and southern habitats, indicating increased adaptability to environmental conditions (Zhang *et al.*, 2023).

The findings in this study are in line with previous research, where hybrids of *M. amblycephala* \times *C.* alburnus exhibited evident growth advantages over their parent species (Zheng et al., 2015). Similarly, intergeneric hybrids of female *M. terminalis* \times male C. alburnus demonstrated a significantly increased growth rate (Guo et al., 2018). Additionally, hybrids of female *M. amblycephala* × male Xenocypris davidi and Clarias anguillaris × Heterobranchus bidorsalis also displayed considerably faster growth rates compared to their parental lines (Diyaware & Onyila, 2014). Internal factors include genetic factors and gender, while external factors encompass biological and environmental conditions such as temperature, acidity level (pH), geographical location, sampling techniques, gonad development, and food availability.

The formation of hybrid Mahseer fish aims to produce individuals with better performance, such as increased weight and specific growth rate, compared to their parents. This occurs because each fish strain has undergone inbreeding and fixed specific genes to varying degrees. By crossing these strains, new genetic materials can be created that are expected to enhance performance compared to the average genetic traits of their parents. The results of these inter strain crosses can generate new gene configurations or reduce the effects of undesirable genes, ultimately leading to fish with improved performance. It is important to note that the genetic factor will depend on the extent to which these gene pairs interact. If there is no interaction between gene pairs, the value will be the same as the sum of the effects of each gene pair. Crossing is a breeding technique

aimed at improving growth rates, delaying gonad maturation, enhancing disease resistance, and creating superior offspring. The research done by Arifin *et al.* (2017) shows that hybridization between black Galunggung gourami and white Galunggung gourami strains results in an average increase in weight, survival rate, specific growth in length and width, final biomass, and productivity. Hybridization has been noted to result in progeny that exhibits superior performance compared to both parent species (Mbiru *et al.*, 2015), amalgamating valuable traits from two species into a singular strain.

Heterosis and heterobelstiosis for survival

The crossbred populations of Mahseer fish after 210 days grow-out period shows not significantly affected (p>0.05) survival rate (Figure 3). The highest value of survival rate is observed in the hybrid of ST with a survival rate of 100%, followed by the population of TS, TD, SS, DD, DS, DT, TT, SD as 96.6%, 93.3%, 93.3%, 93.3%, 90.0%, 90.0%, 90.0%, 76.6% respectively. The ST population demonstrated the highest survival at 100.00%, followed closely by 96.66% for TS and 93.33% for TD, SS, and DD. The lowest survival rate of 76.67% was recorded for the SD population, with a large error bar indicating high variability in the survival rate. Other populations, DS, DT, and TT, maintained survival rates close to 90.00% with relatively small fluctuations. This illustrates an improvement in the survival performance of ST and is in line with the expected outcomes of interspecies hybridization programs.



Figure 3. Survival rate of of 9 crosses (average \pm standar deviation) (p<0.05).

Note : ST (Tor soro \Im x T. tambroides σ), TS (T. tambroides \Im x T. soro σ), SD (T. soro \Im x T. douronensis σ), DS (T. douronensis \Im x T. soro σ), TD (T. tambroides \Im x T. douronensis σ), DT (T. douronensis \Im x T. tambroides σ).

Research of Himawan et al. (2021) show that through a genetic approach, survival rate of fish can be improved by producing synthetic carp population resistant to high environmental ammonia. In contrast to intraspecific crosses, interspecific crosses or hybridization can bring out heterozygosity that was previously suppressed by intraspecific crosses, thereby enhancing fertility and fitness (Falconer & Mackay, 1996). The research results on genetic compatibility (Warwick et al., 1995) indicate that better growth characteristics in hybrids can be associated with a favorable genetic combination from both fish species. In other words, the genetic interaction between male *T. soro* and male *T. tambroides* produces offspring with better survival than others hybrid and purebred. Hybridization can induce strengthening the individual's resistance to the environment, thus resulting in high survival rates. Hybridization can generate new strains that outperform their parents in terms of increased growth rate, survival, sex ratio, and color appearance. Crossbreeding is utilized to increase the likelihood of variation in hybrid offspring (Litsios & Salamin, 2014). This method enables the fusion of the complete haploid genome from one species with that of another. Such inter species genome exchange, especially between genetically distant species, has the capacity to bring about both phenotypic and genotypic changes (Al-Harbi, 2016).

Heterosis and Heterobeltiosis

Growth rate

In general, analysis of mid-parent growth heterosis (Table 1) and heterobeltiosis (Table 2) show that Mahseer hybrid ST have the highest value on final weight and specific growth rate trait.

Strains	Heterosis		
	Final weight	Specific growth	
ST	175.24	73.55	
TS	106.22	-8.10	
SD	57.39	-14.41	
DS	78.50	-2.76	
TD	86.72	7.58	
DT	49.06	-3.11	

Table 1. Growth heterosis of hybrid Mahseer fish population on phenotypic traits

Table 2. Growth heterobeltiosis of hybrid Mahseer fish population on phenotypic

Strains	Heterobeltiosis		
Strains	Final weight	Specific growth	
ST	165.84	54.99	
TS	99.17	-17.93	
SD	46.69	-19.60	
DS	66.37	-8.65	
TD	68.52	-9.06	
DT	34.53	-18.09	

Note : ST (Tor soro Q x T. tambroides \varsigma), TS (T. tambroides Q x T. soro \varsigma), SD (T. soro Q x T. douronensis \varsigma), DS (T. douronensis Q x T. soro \varsigma), TD (T. tambroides Q x T. douronensis \varsigma), DT (T. douronensis \varsigma x T. tambroides \varsigma).

Heterosis in the final weight character of hybrid population of Mahseer fish shows that the ST population produces the highest value at 175.24%, followed by the populations TS, TD, DS, SD, and DT crosses sequentially at 106.22%, 86.72%, 78.50%, 57.39%, and 49.06%. In terms of specific growth, the ST cross exhibits the highest heterosis value at 73.55%, followed by the TD, DS, DT, TS, and SD crosses sequentially at 7.58%, -2.76%, -3.11%, -8.10%, and -14.41%. Furthermore, for the survival rate character, the ST

cross also shows the highest positive heterosis value at 9.09%, followed by the TS, TD, DT, DS, and SD crosses sequentially at 5.45%, 1.82%, -1.82%, -3.57%, and -17.86%.

In general, the heterobeltiosis values for the final weight character were positive, with the highest value achieved in the ST cross at 165.84% and the lowest in the DT population at 35.53%. The analysis results show that all hybrid strainhad positive values for the final

weight character. Furthermore, for the specific growth rate character, the highest heterobeltiosis value in hybrid Mahseer fish was achieved in the ST population at 54.99%, and the lowest in the SD population at -19.60%. For the specific growth rate character, a positive heterobeltiosis value was only achieved by the ST cross, while other hybrid populations had negative values. For the survival rate character, the highest heterobeltiosis value was achieved by the ST hybrid at 7.14%, and the lowest by the SD population at -17.86%. For the survival rate character, the ST hybrid population and its reciprocal TS had positive heterobeltiosis values, while the TD population had a value of zero.

Survival rate

Analysis of mid-parent survival heterosis (Table 3) and heterobeltiosis (Table 4) show that strain ST have the highest value.

The high heterosis and heterobeltiosis values obtained indicate that the influence of non-additive gene effects in the crossbreeding of Mahseer fish is relatively high. High heterosis values depict the compatibility of genes in sharing potential advantages or due to temporary emergence and changes in subsequent generations (Warwick *et al.*, 1995). Heterosis values emerge due to the presence of new gene combinations, which are expected to result in better performance. Outbreeding can induce suppressed heterozygosity compared to inbreeding, thus potentially enhancing fertility and fitness. Positive heterosis effects involve the average performance of a trait in offspring resulting from crossbreeding exceeding the average performance of both parents, while negative heterosis effects involve the average performance of a trait in offspring from crossbreeding being lower than the average performance of both parents. Performance related to production generally exhibits positive heterosis, while reproductive performance generally exhibits negative heterosis effects (Falconer & Mackay, 1996). Several studies on various fish species indicate that the hybrid population heterosis values are better compared to their pure line. Radona et al. (2015) obtained heterosis value for hybridization of seed of T. soro with T. douronensis, with heterosis value for length, weight, and survival rate traits respectively by 40.90%, 116.66%, and 1.26%. The variation in results compared to the previous study is presumed to be due to differences in the male species, specifically Tor douronensis. The study by Ariyanto & Listiyowati (2011) obtained heterosis values of 9.5%, 2.9%, and 15.6% for total length, standard length, and body weight traits in the hybridization of female Bastar gourami fish with male Blusafir gourami. In the crossbreeding of Sangkuriang and Masamo catfish, Nugroho et al. (2015) obtained hybrid heterosis values at the hatchery level of 6.68% for survival rate

Table 3. Survival heterosis of hybrid Mahseer fish population on phenotypic

Strains	Heterosis	
	Survival rate	
ST	9.09	
TS	5.45	
SD	-17.86	
DS	-3.57	
TD	1.82	
DT	-1.82	

Table 4. Survival heterobeltiosis of hybrid Mahseer fish population on phenotypic

Strains	Heterobeltiosis	
	Survival rate	
ST	7.14	
TS	3.56	
SD	-17.86	
DS	-3.57	
TD	0.00	
DT	-3.57	

Note : ST (Tor soro $\mathfrak{P} \times T$. tambroides \mathfrak{F}), TS (T. tambroides $\mathfrak{P} \times T$. soro \mathfrak{F}), SD (T. soro $\mathfrak{P} \times T$. douronensis \mathfrak{F}), DS (T. douronensis $\mathfrak{P} \times T$. soro \mathfrak{F}), TD (T. tambroides $\mathfrak{P} \times T$. douronensis \mathfrak{F}), DT (T. douronensis $\mathfrak{P} \times T$. tambroides \mathfrak{F}).

and 2.79% for seed length increase. The rationale behind hybrid breeding lies in the systematic exploitation of heterosis (Schulthess *et al.*, 2017). Mid-parent heterosis is defined as the difference between a progeny's genetic value and its mid parent value, which is the average of its parent's genetic values.

Overall, the heterobeltiosis values in the ST population indicated higher final weight, specific growth rate, and survival rate compared to the average of its best parent. This condition aligns with the goal of developing hybrid Mahseer fish, which is to obtain a hybrid population capable of growing relatively faster than its parents. This is likely because the ST population possesses dominant alleles that are expressed in the final weight character. The phenomena of heterosis and heterobeltiosis occur due to dominant interactions between genes within a locus, leading to an increase in size or vigor, resulting in better performance than both parents. In this dominance, there are deviations from the genotypic value, where the dominant deviations arise from dominant traits among alleles at a locus and represent interactions between alleles or interactions within a locus. The study by Duong et al. (2022) showed during the larval rearing stage of bighead catfish (Clarias macrocephalus Günther, 1864), most crossbreeds exhibited higher survival rates compared to their parents, resulting in positive values of observed heterobeltiosis and heterosis. However, the variation in growth performance and survival rate among each hybrid indicates that heterosis and heterobeltiosis for growth may not be very common and can depend on the species, genetic background, genetic environment interactions, and life stages (Semeniuk et al., 2019). According to Duong et al. (2022), the absence of heterosis or heterobeltiosis for growth, feed conversion rate, or survival suggests that dominance effects contributing to the phenotypic variance of these traits are likely small.

CONCLUSIONS

The results showed that the ST population have highest phenotypic performance of final weight, specific growth rate, and survival rate. The hybrid population of ST (*Tor soro* x *Tor tambroides*) exhibits better hybrid vigor in final weight, specific growth rate, and survival rate.

ACKNOWLEDGEMENTS

The highest appreciation and special thanks to Research Institute for Fish Breeding (RIFB) Sukamandi for facilitating the field research, as well as to all other parties who helped carry out this research. REFERENCES

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