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HYBRIDISATION OF FEMALE KOI (*Cyprinus rubrofuscus)* AND MALE KAPIAT (*Barbonymus schwanenfeldii)* WITH ARTIFICIAL SPAWNING IN KAMPAR DISTRICT, RIAU

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ABSTRACT

This study aims to evaluate the potential for hybridisation between female koi (*Cyprinus rubrofuscus*) and male kapiat (*Barbonymus schwanenfeldii*) through artificial spawning. The study was conducted at Kampar Ornamental Fish, Kampar District, Riau Province. The method used was a Completely Randomized Design (CRD) with two treatments: P1 (koi × koi) as the control, and P2 (koi × kapiat) as the hybrid treatment, each with three replicates. The parameters observed included fecundity, fertilisation rate (FR), hatching rate (HR), absolute length growth, and survival rate (SR). The results showed that hybridisation produced promising performance in terms of FR (21.99%), HR (55.02%), and SR (41.6%). In conclusion, hybridisation between koi and kapiat fish has the potential to produce superior varieties, although attention must be paid to environmental factors such as temperature and pH.

Keywords: Hybridisation, Koi, Kapiat, Artificial spawning, Fertility,

1. INTRODUCTION

Aquaculture is crucial in supporting food security and economic development for communities in Indonesia, a maritime country with vast water resources. The development of aquaculture technology is key to efforts to increase productivity and quality of fishery products, especially in the freshwater fish sector, which has a growing market demand[1]. One technology that has proven effective in improving the genetic quality of farmed fish is hybridization, which is the crossbreeding of two different fish species to produce offspring with superior characteristics from both parents[2].

Fish hybridization has been widely applied in aquaculture in Indonesia with the primary objective of combining the superior traits of various species, such as fast growth rate, disease resistance, adaptability to the environment, and improved meat quality[3]. This technology is one of the simpler forms of genetic engineering, but it significantly impacts the diversification of aquaculture products.

Koi (*Cyprinus rubrofuscus*) are ornamental fish with high economic value developed through genetic selection from carp (*C.carpio*). The main characteristics of koi fish lie in their attractive colour variations and body patterns, making them a commodity with high aesthetic value in the global market[4]. Through intra-species hybridisation, various koi varieties have been developed to meet the dynamic market demand. Gomelsky & Kaplan[5] confirmed that colour pattern inheritance in normal offspring (amphimictic) depends on the genetic contribution of both parents,

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although the proportion of this contribution is difficult to determine precisely.

On the other hand, the kapiat (*Barbonymus schwanenfeldii*) is a freshwater fish species native to Southeast Asia that has economic potential as a food fish. According to Sumino et al.[6], the kapiat fish is classified as an omnivorous fish with distinctive feeding patterns, depending on the natural food in the water. This species is known for its good adaptability to the aquatic environmental conditions of Indonesia and its relatively fast growth rate, making it a potential candidate for hybridizations programmes.

Although both species belong to the Cyprinidae family, hybridizations between koi and kapiat have not been extensively explored scientifically. Theoretically, family similarities could serve as an initial indicator of the potential success of hybridizations; however, morphological differences, behavioral traits, and habitat preferences may act as limiting factors that require further investigation. Interspecific hybridizations within the Cyprinidae family have been reported to be successful in several species' combinations[7], providing a scientific basis for further research on unexplored species combinations.

This study aims to examine the potential for hybridisation between koi and kapiat in producing new varieties with a combination of superior characteristics from both parents. Specifically, this study will analyse: fertilisation and embryo development from crossbreeding, morphological characteristics of hybrid offspring, particularly in terms of colour patterns and body shape, growth rates and survival rates of hybrids compared to their parents, as well as the adaptability of hybrids to various water quality parameters.

2. RESEARCH METHOD

Time and Place

This study was conducted in October-November 2024 at Kampar Ornamental Fish, Sigha Sungai Street, RT 1/RW 1, Padang Mutung Village, Kampar District, Kampar Regency, Riau Province.

Method

The experimental design used in this study was a completely randomized design (CRD) with two treatments, each consisting of three replicates. The treatments were as follows:

P1 = Hatching of koi x koi (control)
P2 = Hatching of koi x kapiat (hybrid).

Procedures

Container Preparation

The initial preparation that must be done in this study is to prepare containers in the form of aquariums. The containers are arranged first and then cleaned or washed. After cleaning, the containers are placed on the laboratory table and randomised according to the treatment. Next, the containers arranged on the table are filled with water to 10 cm in each container and equipped with aeration as an oxygen supply and a filter as a water reservoir throughout the research.

Maintenance Media

In this study, the medium used was water from a borehole. The water was put into a reservoir and aerated. The water medium was settled for 1 day before being used as a test medium. Then, the water was distributed into each hatching container to a height of 10 cm.

Spawning of Broodstock

The first step is to prepare koi fish eggs through artificial spawning of mature gonads. Before spawning, the koi broodstock are starved. Spawning is carried out by injecting the hormone ovaprim at a dose of 0.5 cc/kg body weight. The injection is administered at 00:00 WIB, and the stripping process is carried out the following morning at 08:00 WIB.

Fertilization, Incubation, and Hatching

Artificial insemination is performed by collecting sperm from the male parent using a syringe, which is then temporarily stored in a glass containing ice. The female parent is then stripped, and the sperm is mixed evenly with the eggs in a bowl. The fertilized eggs are weighed and placed in a prepared aquarium.

After fertilization, the eggs are weighed, and the next step is to incubate the eggs in a prepared medium and container. The eggs are spread into a container containing sperm and stirred using chicken feathers. During Hatching, the water is changed on the first day to reduce foam and maintain quality. The hatching time for koi × kapiat eggs is approximately 36–40 hours until they hatch completely.

Fecundity

Fecundity was calculated based on Kalhoro et al.[7]. The formula used was:

$$F = (W_g/W_s) \times N$$

Description:

F	=	Fertility
W_g	=	Gonad weight (g)
W_s	=	Sample weight (g)
N	=	Number of eggs in sample
F	=	(Wg/Ws) x N

Fertilization Rate (FR)

To determine the degree of fertilisation of fish eggs, the formula by Ishaqi et al.[8] can be used as follows:

$$FR\ (\%) = \frac{Po}{P} = 100\%$$

Description:

FR	=	Degree of egg fertilization (%)
P	=	Number of sample eggs
P_o	=	Number of fertilised eggs

Hatching Rate (HR)

According to Efrizal in Larasati et al.[9] to calculate egg hatchability, the formula is used as follows:

$$HR\ (\%) = \frac{Pt}{Po} \times 100\%$$

Description:

HR = Hatching Rate (%)
P_t = Number of eggs hatched
P_o = number of fertilised eggs

Absolute Length Growth

Absolute length growth was obtained using the formula by Arunde et al.[10] as follows:

$$P = Pt - Po$$

P	=	Absolute length growth (cm)
P_t	=	Average length of fish at the end of cultivation (cm)
P_o	=	Average length of fish at the beginning of cultivation (cm)

Survival Rate

The method used was to calculate the Survival Rate (SR) using the formula by Nurbaeti[11] as follows:

$$SR\ (\%) = \frac{Nt}{No} \times 100\%$$

Description:

SR	=	Survival rate (%)
N_t	=	Number of fish at the end of the study (fish)
N_o	=	Number of fish at the beginning of the study (fish)

3. RESULT AND DISCUSSION

Hybridisation of Koi and Kapiat

Hybridisation between female koi and male kapiat was done using artificial spawning techniques to overcome natural reproductive barriers between different species. The success of hybridizations was indicated by fertilization following the injection of ovaprim hormone into the female parent, followed by the stripping process and manual mixing of gametes. After the eggs and sperm were mixed, egg colour and texture changes were observed as signs of fertilization.

During incubation, fertilized eggs showed embryonic development, including initial cell division (cleavage), somite formation, and embryo movement before hatching. Hatching occurred within 36–40 hours, and the hatched larvae exhibited mixed morphology from both parent species, such as the characteristic colour of koi with a body shape resembling kapiat.

This is an early visual indication that the hybridizations process was successful. Differences in fertilization rate and survival rate between the hybrid and control groups were also used as supporting parameters for the success of hybridizations. The results showed that although the fertilization rate in hybrids was lower than in the control group, some eggs could develop into larvae, indicating the successful formation of hybrid embryos.

Thus, this study successfully demonstrated that hybridisation between koi and kapiat is possible through artificial spawning techniques, although fertilisation efficiency and survival rates still need to be optimised.

Measurement Parameters for Hybridisation of Koi and Kapiat Fish

The egg fertility results for koi and kapiat can be seen in Table 1.

Table 1. Fertility of koi and kapiat

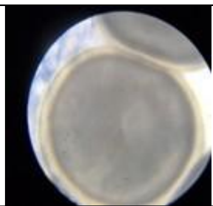
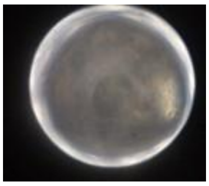
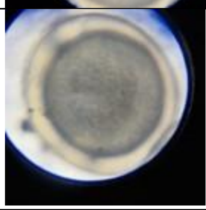
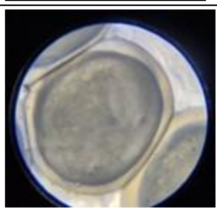
Spawning	Koi Fish Strain	Initial weight of parent (g)	Final Weight of Parent (g)	Fecundity (Grain)	Length (cm)
1	Kohaku	300	219	640	23
2	Sanke	310	215	622	25
3	Shiro	317	213	635	26

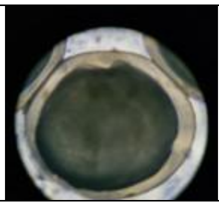
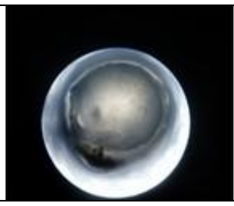
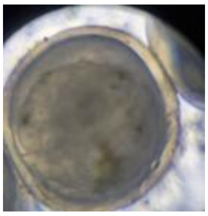
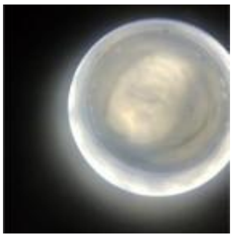
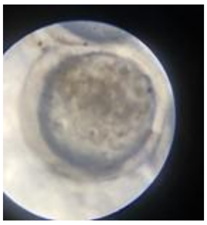
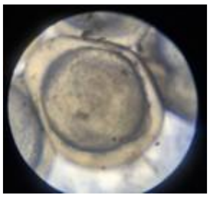
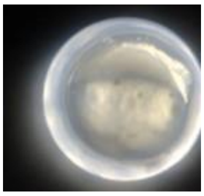
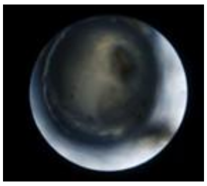
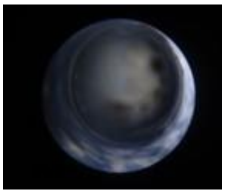
Table 1 shows the fecundity results of 3 female koi fish of the kohaku, sanke, and shiro varieties and three female kapiat, with 640 eggs collected. Changes in the weight of the broodstock are related to energy allocation for reproduction. According to Meretsky et al.[13]; Habibi et al.[14] good nutrition supports gonadal maturation and fecundity, while low-quality feed can reduce egg quality and quantity.

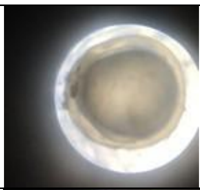
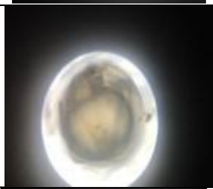
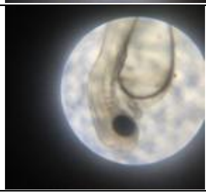
Embryogenesis of Koi x Kapiat (Hybrid) and Koi

The embryogenesis of koi x kapiat (hybrid) and koi fish can be seen in Table 2.

Table 2. Embryogenesis of koi x kapiat (hybrid)

No	Phase Development	Minute	Hybrid Embryo Development	Embryonic development of koi	Description
1	Cleavage	0			At 0–30 minutes, the fertilised eggs form small yellow clusters of blastomeres.
		10			
		20			Cell division occurs from the 20th minute to the 40th minute, starting from 2 cells, 4 cells, 8 cells, 16 cells, until the morula phase.

	40			
	60			At minute 60, cell division occurs, resulting in cells doubling in number until many cells are formed. Inside the cells are small cavities called segmentation cavities.
Morula	120			From the 80th minute to the 120th minute, the egg enters the morula phase, the final product of cleavage, when the blastomeres number around 16-32 cells.
Blastula	240			At 240 minutes, the egg develops into the blastula phase, which means that the blastomeres divide several times and form small blastomeres, so that the place in the morula phase that was originally solid becomes a space called the blastocoel, which is covered by the blastoderm, and the epiblast covers the outer side.
Gastrula	360			This phase begins with forming six somites and the optic primordium, which then develop into 10–20 somites. The eyes begin to round, but the heart and blood vessels have not yet formed. Epiboly occurs, which is the expansion and closure of the yolk sac by the blastoderm towards the blastopore, accompanied by the germ ring and shield formation. Epiboly movement occurs between 50% and 90%,

				and the rotation of cells marks the transition to the gastrula phase. In the final stage of gastrulation, embryonic development is characterised by the tail bud (tail bud) appearance.
Sugmentasi	720			From minute 720 to minute 2160, the eyes become rounder, the tail becomes more visible, and the body size increases. Twenty somites are formed.
	1440			
	1800			
	2160			
Larva	2880			At minute 2880, the eggs hatched into transparent larvae. The spine, tail, eyes and mouth were visible at this stage.

An embryo is an organism's development stage from fertilization to hatching. Embryology studies the growth of embryos, while embryogenesis encompasses the processes of their formation and development. During embryogenesis, a series of cell divisions, differentiations, and organogenesis occur, which are greatly influenced by the genetic stability and chromosomal compatibility of the male and female gametes.

The differences in embryogenesis between hybrid embryos and normal embryos generally lie in the stability of their development and viability. Development generally proceeds stably in normal embryos (intraspecific) because there is no genetic conflict between the parental gametes. In contrast, in hybrid embryos (interspecific hybrids), especially between distantly related species, disturbances are often found in the early stages of embryogenesis, such as incomplete cell division, developmental delays, and even failure to hatch (embryonic abortion).

Hybridisation can cause genetic expression mismatches, such as an imbalance in chromosome numbers or incompatible genes between two species. This can result in embryonic malformations, metabolic disorders, or imbalances in synthesising important proteins during development. For example, in a study by Fariedah et al.[15], the embryogenesis of crosses between female *C. carpio* fish and male fish from a different species showed higher levels of embryonic abnormalities than the control group. Meanwhile, embryonic development can proceed relatively normally in hybridizations within the same genus and with high genetic compatibility, although it still carries a higher risk than non-hybrid embryos.

Thus, studying embryogenesis in hybrid fish is vital for understanding the hybridisation process and predicting the resulting offspring's survival rate.

Fertility of Koi and Kapiat

The results of the Fertilisation Rate (FR) calculations for koi fish and fish can be seen in Table 3.

Table 3. Fertilisation rate of koi and kapiat

Description	P_1U_1 Controls	P_1U_2 Controls	P_1U_3 Controls	P_2U_1 Hybrid	P_2U_2 Hybrid	P_2U_3 Hybrid
Number of fertilized eggs	1470	652	126	532	572	312
Total number of eggs	3250	4032	2752	3283	4081	4076
FR (%)	45.23	16.17	4.58	16.21	14.01	7.66

In Table 3, Fertilisation Rate is the percentage of eggs successfully fertilised during spawning. The average FR in treatment P1 was 21.99% and in P2 was 12.62%, with the highest value in P1U1 (3,250 eggs). FR is influenced by egg quality, sperm quality, and water conditions such as temperature[16-17].

The low fertilisation rate, particularly in treatment P2, may be caused by physiological incompatibility between male and female gametes due to species differences, thereby inhibiting fertilisation. In hybridisation, fertilisation success is greatly influenced by genetic compatibility between parents, which includes the compatibility of gamete structure and function. Other factors that influence this include sperm viability and oocyte quality, and artificial insemination techniques such as mixing time and sperm-to-egg ratio.

Similar research by Susanti et al.[18] on hybridisation between carp (*C.carpio*) and nilem (*Osteochilus hasselti*) also reported low fertilisation rates of only around 15–25%, which was caused by species differences and obstacles in the gamete synchronisation process. However, in some cases, such as the hybridisation between local catfish and dumbo catfish (*Clarias* sp), the fertilisation rate can exceed 70% because the two species are still within the same genus and are genetically closer[19]. This indicates that the greater the genetic distance between parents, the higher the likelihood of gamete incompatibility, leading to a decrease in fertilisation success rates.

Hatching Rate Koi and Kapiat

The results of the hatching rate calculations for koi and kapiat from two treatments with three replicates are presented in Table 4.

Table 4. Hatching rate of koi and kapiat

Description	P_1U_1 Controls	P_1U_2 Controls	P_1U_3 Controls	P_2U_1 Hybrid	P_2U_2 Hybrid	P_2U_3 Hybrid
Number of eggs hatched	1470	407	1283	336	120	253
Number of fertilized eggs	3250	652	126	532	572	312
HR (%)	58.43	62.42	10.18	63.00	20.98	81.09

In Table 4, the average Hatching Rate (HR) in treatment P1 was 43.67% and in treatment P2 was 55.02%, with the highest result in P1U1 (1,470 eggs hatched from 3,250 fertilised eggs). HR variation is influenced by internal factors such as egg and sperm quality and external factors such as temperature, pH, dissolved oxygen, and ammonia[20]. Optimal temperature accelerates hatching, while low temperature can inhibit or prevent it.

Absolute Length Growth

The results of the calculations of the absolute length growth of koi fish and kapiat fish with two treatments, each consisting of three replicates, can be seen in Table 5.

Table 5. Absolute length growth

Description	P_1U_1 Controls	P_1U_2 Controls	P_1U_3 Controls	P_2U_1 Hybrid	P_2U_2 Hybrid	P_2U_3 Hybrid
Average final length (mm)	21.9	26.4	28.5	20.3	28.8	25.9
Average initial length (mm)	6.4	6.6	5.8	6.3	7	6.8
Results (mm)	15.5	19,4	22.7	14	21.4	19.1

The absolute length growth calculations for koi and kapiat in two treatments with three replicates show that the highest average length was found in treatment P1U3 at 22.7%, while the lowest was in treatment P2U1 at 14%. Growth measurements were taken by sampling 10 fish four times, once every week. These growth differences are influenced by the fish's osmoregulatory capacity and environmental factors such as temperature and pH. The slow growth in treatment P1U2 is suspected to be caused by high mortality rates due to stress triggered by relatively high ammonia levels.

Survival Rate

The results of the Survival Rate (SR) calculation for koi fish and kapiat with two treatments, each consisting of three replicates, can be seen in Table 6.

Table 6. Survival Rate

Description	P_1U_1 Controls	P_1U_2 Controls	P_1U_3 Controls	P_2U_1 Hybrid	P_2U_2 Hybrid	P_2U_3 Hybrid
Final number of studies	300	21	86	88	95	51
Initial number of studies	1470	407	1283	336	120	253
SR (%)	20	5.1	6.70	26	79	20

The results of survival rate calculations for koi and kapiat in two treatments with three replicates show significant differences. In treatment P1, the highest average SR reached 10.6%; in P2, the highest SR of 41.6%. Conversely, the lowest SR was found in treatment P1U2 (control) with a value of 5.1%. These differences in survival rates indicate variations in conditions or treatments that influence the survival rate of fish larvae.

The low SR in the control treatment compared to the hybrid treatment may be due to genetic resistance and environmental adaptation factors. Hybridization between female koi and male kapiat may produce offspring with hybrid vigour, i.e., improved performance compared to their parents, including resistance to environmental stress, disease, or suboptimal water quality. Conversely, seeds from the control group that did not undergo hybridizations may be more susceptible to unfavorable environmental conditions, leading to higher mortality rates. This suggests that hybridizations can potentially enhance the survival rate of fish seeds by improving their offspring's physiological and adaptive qualities.

4. CONCLUSION

Research shows that hybridisation between koi and kapiat can produce good performance potential, as indicated by positive parameters of fecundity, fertilisation (FR), hatchability (HR), length growth, and survival rate (SR), as long as water quality is maintained. The success of hybridisation is greatly influenced by environmental conditions, especially temperature and pH, which support the osmoregulation ability of fish. The low survival rate in the P1U2 treatment was caused by stress due to high ammonia levels, while the high SR in other treatments reflected optimal water conditions.

It is recommended that research be continued to produce new varieties by attempting hybridizations between koi fish and other species. This process must be carried out carefully to prevent negative impacts on the ecosystem or local species, especially if the hybrid fish are released into the wild. Further research is also important to evaluate the potential ecological risks that may arise outside of the aquaculture environment.

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