Reproductive performance of crossbreeding between local female and imported male koi at CV. Indokoi Malang

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Abstract. The high demand for koi fish must be supported by an adequate supply of fry, both in quantity and quality. High-quality fry comes from superior broodstock. This study aimed to evaluate the quantity, type, and quality of fry produced by crossbreeding local and imported broodstock. A descriptive quantitative method was used, with natural spawning at a ratio of 1:3 (female), involving Shiro F1, local Shiro, local Showa, and imported Ginrin Shiro. Results showed a fecundity of 103,800 eggs, with fertilization, hatching, and survival rates of 84%, 93%, and 74.7%, respectively. The first selection yielded 79% high-quality fry, while the second selection produced 10% high-quality fry. Showa fry dominated the second selection, comprising 84% of that batch and 9.2% of the total fry, while Shiro fry made up 16% of the second selection and 1.7% of the overall fry. The highest daily growth rate occurred in the first week (4.6%/day), while the lowest was in the second week (2.08%/day). The highest specific growth rate was also in the first week (36.43%/day), with the lowest in the fourth week (5.61%/day). Water quality remained suitable for koi fry survival throughout the study.

1 Introduction

The koi fish (*Cyprinus carpio*) is a highly valued freshwater ornamental fish, prized for its unique patterns and colors, which have garnered significant popularity among enthusiasts [1]. According to [2], the beauty of koi lies in its physical appearance and behavior. To meet the high market demand for koi, ensuring an adequate supply of high-quality fry is crucial. High-quality fry is produced by superior broodstock, characterized by distinct, non-gradated colors, balanced patterns, and readiness for spawning. Effective broodstock management involves careful selection. The selection of broodstock ready for spawning is essential for obtaining many high-quality eggs [3]. Criteria for broodstock selection before spawning include color brightness, body pattern, gonad maturity, and overall health [4]. Both imported and local broodstock are used depending on the needs of the breeders. Currently, the demand for koi is primarily met by imported koi, which are considered to have better quality than

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local koi [4]. Imported broodstock are known for their sharp colors, more enormous and more prominent eyes, elongated bodies, and downward-protruding mouths.

In contrast, local koi have colors that fade with age, normal-sized eyes, rounder bodies with slight bulging, and forward-protruding mouths. Spawning using imported broodstock can yield high-quality fry. Spawning is conducted naturally by placing male and female broodstock in the same pond. A higher number of male broodstock is used to ensure the eggs are optimally fertilized by the male's sperm [5]. The ratios used in spawning can vary (female) from 1:1, 1:2, to 1:3. In this study, imported and local broodstock were used to produce a large quantity of high-quality fry. The study aims to evaluate the quantity, type, and quality of fry resulting from the crossbreeding of imported and local Showa broodstock, providing valuable insights for breeders in subsequent spawning efforts to produce superior fry with continuous availability.

2 Materials and Methods

2.1 Time and Location

This research was conducted from July to August 2023 at CV. Indokoi Malang. The koi egg hatching was examined in the Fisheries Laboratory of the Faculty of Agriculture and Animal Husbandry, University of Muhammadiyah Malang.

2.2 Materials and Equipment

The materials used in this research included ready-to-spawn male and female koi broodstock, koi eggs, koi fry, boiled chicken egg yolk, Tubifex worms, and commercial feed. The equipment used included concrete ponds, spawning mats (kakaban), aeration systems, pumps, filter tanks, pond filters consisting of bioballs, coral rocks, oyster shells, nylon nets, fishing nets, pH meters, dissolved oxygen (DO) meters, rulers, analytical balances, nets, plastic containers, inlet pipes, and outlet pipes.

2.3 Research Method

This study utilized a descriptive quantitative research method. The quantitative approach involved data collection followed by interpretation and presentation of the results [6]. The steps in descriptive research include gathering information about the research problem, explaining the objectives, planning the approach, and collecting data for report writing. Data collection was carried out through observation, interviews, and active participation in the activities of CV—Indokoi Malang, as well as through a literature review. Data collection began at the onset of spawning and continued through the second selection, with weekly sampling conducted to monitor growth and water quality during the study.

2.4 Broodstock Management

Broodstock management involved the selection of broodstock that were ready to spawn, had mature gonads, and were of sufficient age—females over two years and males over one year. The selected broodstock had a perfect, non-deformed body shape, sharp and non-gradated body color, and a well-defined pattern from head to tail without blockiness. Before spawning, the broodstock was maintained by feeding them to support growth and maintain color. Feed was provided 2-3 times daily, in the morning and evening, with 200 grams per feeding. The commercial feed was fermented with probiotics and vitamins to ensure easy digestion.

Broodstock was transferred from the spawning pond in the evening. The transfer process began by netting the broodstock, which was then placed into plastic bags filled with water and supplemented with oxygen. Different types of broodstock could be packed together, but males and females were kept separate. However, female broodstock containing eggs could be packed with males if the distance between the broodstock pond and the spawning pond was short. Transfers were conducted in the evening when the temperature was lower, reducing stress on the fish. After spawning, the koi broodstock was quarantined and then placed into a broodstock pond to prevent the spread of pathogens.

2.4.1 Fecundity

Fecundity refers to the total Number of eggs produced from spawning. Fecundity was measured by weighing the broodstock before and after spawning. This approach was used because the Number of eggs produced naturally is very high, and weighing the difference in Weight simplifies the process. Fecundity is influenced by the nutrition provided for gonad development and the environment. The formula for calculating fecundity according to [7] is as follows:

$$F= \frac{Wg}{Ws} \times N$$

- F = Fecundity
- Wg = Total Weight of gonads
- Ws = Weight of gonad sample
- N = Number of eggs in the sample

Fertilization Rate (FR):

The fertilization rate is the percentage of fertilized eggs out of the total eggs produced during spawning. It is calculated by randomly sampling and observing the eggs. The FR is influenced by egg quality, sperm quality, and water quality, including temperature and pH. According to [8], the fertilization rate can be calculated using the following formula:

$$FR\ (\%)= \frac{Fertilized\ eggs}{Total\ of\ eggs} \times 100\%$$

Hatching Rate (HR):

The hatching rate is the percentage of fertilized eggs that successfully hatch. The HR is influenced by the ability of sperm to fertilize eggs; the more eggs fertilized, the higher the hatching rate. According to [8], the hatching rate can be calculated using the following formula:

$$HR= \frac{Number\ of\ larvae}{Fertilized\ eggs} \times 100\%$$

Survival Rate (SR):

The survival rate is the percentage of fish that survive over a specific period compared to the Number at the beginning of the rearing process. The calculation is made using a sampling method. According to [9], the survival rate is calculated as follows:

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

Probability (P):

Probability refers to the likelihood of a specific event occurring out of all possible events. According to [9], the probability is calculated using the following formula:

$$P = \frac{n(A)}{n} \times 100\%$$

Koi Fry Selection:

The selection of koi fry is conducted twice before they are introduced into the rearing pond. The first selection is performed seven days after egg hatching, and the second selection is conducted 1 month after the first selection. The first selection aims to sort out red or "kopyrok" and black fry. The second selection differentiates between fry types and assesses their quality.

Daily Growth Rate:

The daily growth rate is the change in fish length over time. According to [8], the daily growth rate is calculated using the following formula:

$$\text{Daily growth rate} = \frac{lnLt - lnLo}{t} \times 100\%$$

Specific Growth Rate (SGR):

The specific growth rate is the daily increase in fish weight during the study period. According to [8], the specific growth rate is calculated as follows:

$$SGR = \frac{(lnWt - lnWo)}{t} \times 100\%$$

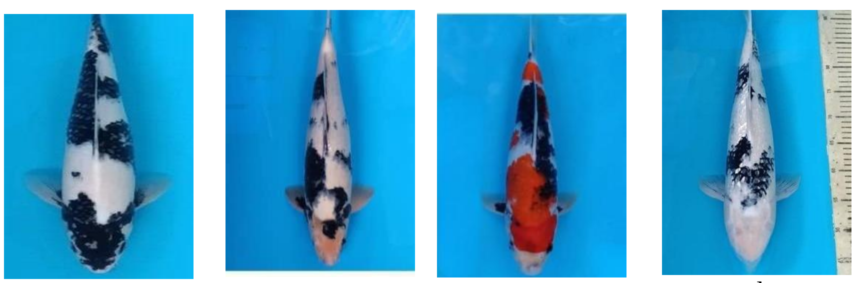
3 Results and Discussion

The criteria for selecting koi parents include sharp coloration, consistent patterns from head to tail, and readiness for spawning. High-quality parents ensure the production of high-quality offspring, while poor-quality parents result in fewer and lower-quality offspring [11]. Additionally, koi parents must exhibit strong disease resistance and superior growth. The criteria for selecting koi parents for breeding are detailed in Table 1.

Table 1. Koi parental criteria

No	Characteristic	Ref. [12]
1	Female >2 years old, Male > 1 year old	Female >2 years old, Male > 1 year old
2	Body weight 2-4 kg	Female Weight: 1-4 kg, male: 1-4 kg*
3	Female body: proportional, pregnant Male body: proportional, pelipis kasar	Female body: proportional, bulky, big back Male body: proportional, Bulky, significant bac big back
4	Female color: bright, clear, no gradation Male color: clear, yellowish	Female color: solid, not faded, no gradation male color: bright, solid, not faded
5	Size > 50 cm	Size 35-45

For female koi ready for spawning, the criteria include a bulging belly, rough operculum, and the release of yellow fluid when pressed. For males, the criteria are agile movement, a rough operculum, a slender body, and sperm release when pressed. Mature male parents have rough bodies and release milky white fluid (sperm) when stripped. Mature parents produce offspring of high quality and quantity [13]. The parents selected for spawning are shown in Fig.1.



a b c d

Fig. 1. Strain of parental (a) Shiro F1, (b) Shiro, (c) Showa, (d) Imported Shiro Ginrin

Based on Fig. 1, the female parent is of the Shiro F1 strain, which will be crossed with three male parents: a local Shiro, a local Showa, and an imported Ginrin Shiro. The imported Ginrin Showa parent was sourced from Taniguchi Koi Farm. The Shiro strain has a white base color with black patterns, while the Showa strain has a black base color with red and white patterns. The Showa strain's base color is black with white and red spots, whereas the Shiro strain's is white with black spots [13]. Ginrin koi are characterized by their transparent white scales, resembling shards of glass.

3.1. Spawning Process

This study conducted the spawning process naturally by placing male and female koi parents into a single pond equipped with kakaban and aeration during the evening, as fertilization occurs at night. The best time for releasing parents is in the evening when temperatures are lower [14]. Kakaban serves as a substrate for the eggs to attach [15]. The parent ratio was 1:3 (female), with Shiro F1 crossed with local Shiro, local Showa, and imported Ginrin Shiro. The spawning process begins with the male chasing the female, leading to fertilization as the female releases eggs and the male releases sperm onto the prepared kakaban. The parents are removed after spawning is complete, indicated by a flattened and concave belly. The removal of the parents is done gently, and they are then placed in plastic bags and transferred to a quarantine aquarium. Post-spawning quarantine is performed to prevent pathogens from being introduced into the parent pond [16]

3.2. Fecundity

Fecundity was determined by weighing the parents before and after spawning to estimate the Weight of the gonads. In this study, the initial Weight of the parent was 1.8 kg (1,800 grams), and the final Weight was 1.5 kg (1,500 grams), resulting in a gonad weight of 300 grams. The fecundity value in this natural spawning study was 103,800 eggs, which is relatively high. This finding is consistent with [15], who reported fecundity values of 135,000 eggs with a gonad weight of 350 grams in the first spawning and 150,000 eggs with a gonad weight of 450 grams in the second spawning. Both internal and external factors influence fecundity. Internally, it is affected by the species and genetics of the fish, while externally, it is influenced by temperature, feed, and habitat [16]. Fecundity is closely related to the metabolic processes and energy needed for egg development, such as age, size, and spawning time [17]. The Weight of the female parent also affects fecundity, as it reflects the gonad weight [15].

3.3. Fertilization Rate

In this study, the fertilization rate was 84%, considered good. A sample of 150 eggs was taken, and 127 eggs were successfully fertilized. Fertilized eggs, or regular eggs, are characterized by their transparent appearance with a yellow center. In contrast, abnormal eggs are milky white. A fertilization rate above 50% is considered high, 30-50% is medium, and below 30% is low [18]. Only complete egg maturation can help fertilization. Egg maturation is influenced by hormonal activity, which assists in the reproductive process. Additionally, the fertilization rate depends on sperm quality, the handling system, and the time required for egg fertilization [19]. The microscopic appearance of the eggs is shown in Fig. 2.

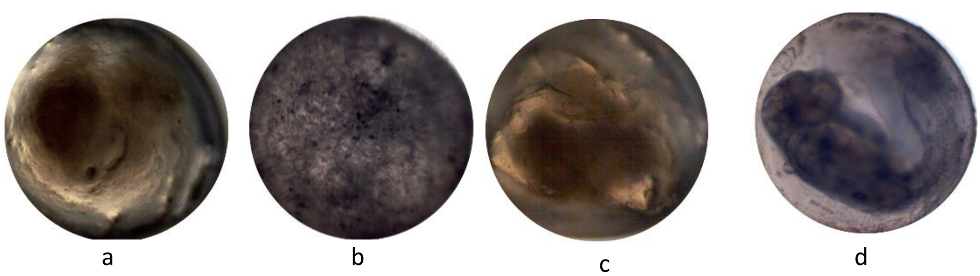


Fig. 2. Eggs condition (a) normal, (b) abnormal, (c) 14-hour egg, (d) 18-hour egg

Based on Fig. 2: (a) Normal eggs are perfectly round and yellow, indicating the presence of yolk that will develop into larvae. (b) Abnormal eggs are not perfectly round and white because central yellow is absent. (c) At 14 hours, the larvae begin to appear. (d) At 18 hours, the larvae start to move and hatch at 20 hours. Fertilized eggs are yellow and precise, while unfertilized eggs are milky white. Fertilized eggs will hatch into larvae, while unfertilized eggs will remain pale white. Koi eggs are round and yellow, with a 1.5–1.8 mm diameter, and weigh 0.17–0.20 mg [17].

3.4. Hatching Rate

This study's hatching rate was 93%, which is considered good. This result is influenced by the fertilization rate, with 127 eggs successfully fertilized and 119 eggs hatching into larvae. Koi eggs attached to kakaban will hatch within 2-3 days, depending on temperature, oxygen, and pH levels. Temperature influences the hatching rate; higher temperatures result in faster hatching. The optimal temperature for fertilization is 27°C, and hatching occurs at 38°C [20]. Temperature affects average growth and determines the hatching time and embryo and fry development. High water temperatures cause premature embryos, while low temperatures delay hatching due to the embryo being retained within the eggshell. The ideal water pH for hatching is 6.5-8.5 [21]. Another factor affecting the hatching rate is mortality, where overlapping eggs lack oxygen, leading to death [12].

3.5. Survival Rate

The survival rate of koi larvae in this study was 74.7%, which is considered moderate. The SR is influenced by the survival of the larvae, which are prone to high mortality due to their limited tolerance to new environments. Temperature affects activities such as respiration, growth, and reproduction. Additionally, water quality is influenced by the availability of food. The early life of larvae is highly dependent on the maternal energy reserves the parents provide [11]. Newly hatched larvae carry food reserves like yolk, which they use as food sources [12]. During the larval stage, koi undergo a critical phase, requiring time to adapt to new feeding patterns essential for survival. Natural feed contains high protein, is easy to obtain, digestible, relatively inexpensive, and does not easily contaminate the rearing media [19]. The larvae require natural feed with high protein content. In this study, natural feeds provided included egg yolk and silk worms.

3.6. Fry Selection

After hatching, the selection of fry from the Showa crossbreeding can begin when they are 7 days old. The selection data is presented in Fig. 3.

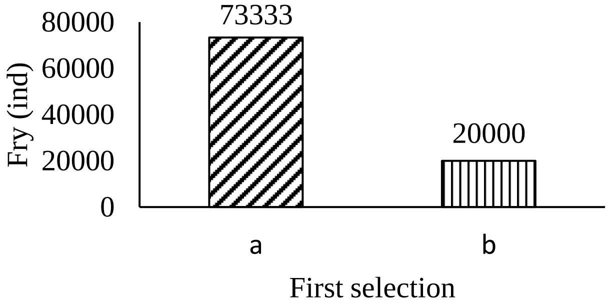


Fig. 3. First Selection Data (a) Qualited/selected fry (b) Low-Quality/non-selected fry

Based on the first selection data, 73,333 quality fry were produced, representing 79% of the eggs successfully hatched. These fry were then further cultivated. The Number of low-quality or sorted fry amounted to 20,000, or 21% of the total fry. Quality fry was identified as black fry, while low-quality fry, commonly known as "kopryok," was identified by its red color. The selection process continued when the fry reached 30 days of age, with the second selection results shown in Fig. 4.

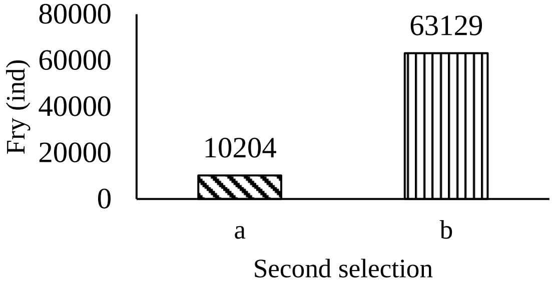


Fig. 4. Second Selection Data (a) Qualited/selected fry (b) Low-Quality/non-selected fry

Based on Figure 5, the second selection resulted in 10,204 quality fry, representing 13% of the total fry from the first and 10% of the overall fry. These quality fry consisted of Showa and Shiro varieties. The low-quality fry totaled 63,129, accounting for 86% of the fry from the first selection and 67%. The low-quality or sorted fry criteria included plain coloration, kopryok, block patterns at a single point or over the entire body, and physical deformities. Plain fry could be sold immediately as they have a low market value if kept for cultivation.

Meanwhile, the quality of the fry was transferred to ponds for further cultivation. The criteria for high-quality koi fry include perfect body shape, sharp and clear coloration without gradation, and a non-blocked pattern. High-quality koi have an ideally shaped body, vivid and consistent coloration without gradation, calm yet agile movement, and live in groups. Showa fry are characterized by their black bodies [13].

3.7. Probabilitas

The probability in this study refers to determining the likelihood of different types of koi offspring resulting from the crossbreeding of local and imported Showa strains based on data collected after the second selection. The data on the fry types is shown in Fig. 5.

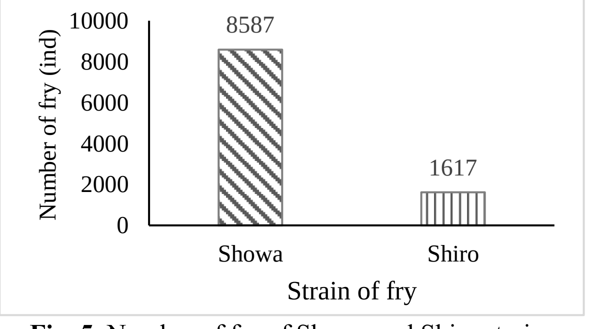


Fig. 5. Number of fry of Showa and Shiro strains

Based on the data above, the probability of obtaining Showa fry is 8,587 fish, or 84% of the total quality fry from the second selection and 9.2% of the overall fry. The Number of Shiro fry is 1,617 fish, 16% of the total quality fry from the second selection and 1.7% of the overall fry. The dominance of the Showa type indicates that the parents used, especially the Showa male, were superior, leading to high-quality and abundant offspring. Superior parents will consistently produce good fry. High-quality koi result from superior parents, excellent fry, and a supportive environment and diet [12].

3.8. Daily Growth Rate

Length is an indicator of the daily growth rate of fish, with growth indicated by differences in size from the beginning to the end of rearing. The graph of the daily growth rate of koi fry is shown in Fig. 6.

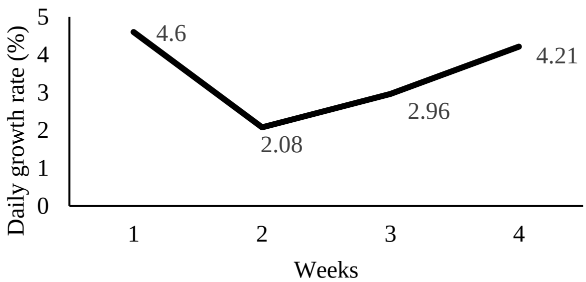


Fig. 6. Daily growth rate of fry

Based on the data above, the average daily growth rate of koi fry over 30 days of research was highest in the first week at 4.6%/day and lowest in the second week at 2.08%/day. The decline in daily growth from the first to the second week was due to a change in diet from natural to commercial feed. The survival of larvae occurs in two phases: critical and transitional. The first phase begins at the start and ends with the consumption of yolk, while the second phase starts when the larvae begin seeking external food [11]. In the third week until the end of the study, the daily growth rate increased because the fry could utilize the feed effectively. In the first week, they utilized yolk (food reserves), boiled egg yolk, and tubifex worms, which are easy to consume and highly nutritious. Yolk contains protein and fat as an energy source [18]. Tubifex worms are high in protein and easy for koi fry to digest. Tubifex worms yielded the highest value in koi larvae treatment, at 56%. The commercial feed used after natural feed is essential for growth and color quality [22].

3.9. Specific Growth Rate

Specific growth rate refers to the percentage increase in fish weight per day. The graph of the specific growth rate of fry resulting from the crossbreeding of Shiro and Showa is shown in Fig. 7.

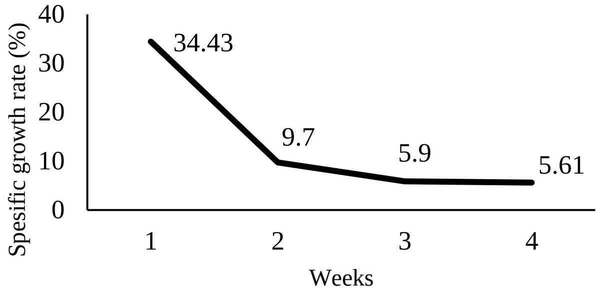


Fig. 7. Specific growth rate of fry

Based on the data above, the highest average specific growth rate was in the first week at 36.43%/day, and the lowest was in the fourth week at 5.61%/day. The decrease in specific growth rate is attributed to the fry utilizing yolk and natural feed, such as boiled egg yolk and tubifex worms, which have high protein content and are well-suited to their digestion, allowing nutrients to be absorbed fully. This contrasts with the second week through the end of the study, where commercial feed was used, requiring fry to adapt to utilize it for survival. The specific growth rate indicates the fish's ability to convert nutrients from feed into stored body energy. The survival of larvae depends on their adaptation to their environment, age, food availability, and water quality. Efficient feed utilization means that fish can convert feed into growth while minimizing energy needs [23].

3.10. Water Quality

Temperature, pH, and dissolved oxygen (DO) measurements were taken weekly. The water quality measurements during the study showed that the ranges obtained were within the optimal limits for koi fry survival. The water quality measurement results are presented in Table 2.

Table 2. Water quality data at the research location

Parameter	Weeks				Optimal [24]
	1	2	3	4	
Temperature (ºC)	25.9-27.8	25.9-27.9	26.8-27.2	26.9-27.9	20-28
pH	8.1-8.5	8.3-8.5	8.3-8.5	8.3-8.4	6.5-8
DO (mg/L)	5.2-5.8	5.2-5.9	5.1-5.9	5.1-6/1	>5

Note: SNI 7734:2017

Based on the temperature measurements during the study, the range was ideal for koi growth, with an average temperature of 25.9-27.9°C. The optimal temperature for koi fry growth is 22-24°C [20], while the optimum range for the survival of organisms is 25-30°C. Temperature plays a crucial role in aquatic environments as it affects appetite, growth, metabolism, and the dissolved oxygen levels in the water. Suboptimal temperatures can negatively impact fish's metabolic processes, making it a critical factor in aquaculture [19].

The pH levels recorded during the study averaged between 8.1 and 8.5, which is still within the optimal range for the survival of koi fry. The ideal pH for koi cultivation is between 6.5 and 8.5 [25]. However, pH levels that are too low or too high can disrupt fish life, with a pH of 4 (acidic) and pH of 11 (alkaline) being fatal to fish [26]. The study's average dissolved oxygen (DO) levels ranged from 5.1-6.1 mg/L. Dissolved oxygen is crucial for organic and inorganic waste's respiration, growth metabolism, and aerobic oxidation processes. High dissolved oxygen levels indicate good water quality. Poor oxygen quality can lead to a decline in fish health, making them more susceptible to diseases or parasites [26].

4 Conclusion

Based on the research findings, it can be concluded that the spawning process with a 1:3 (female) ratio using a Shiro F1 female parent with male parents of local Shiro, local Showa, and imported Ginrin Shiro resulted in a fecundity of 103,800 eggs, with a fertilization rate (FR) of 84%, a hatching rate (HR) of 93%, and a survival rate of 74.7%. In the first selection, 73,333 fry, or 79% of the total larvae, were identified as high quality, while 20,000 fry, or 21%, were deemed low quality. The second selection yielded 10,204 high-quality fry, accounting for 14% of the second selection's total and 10% of the overall fry population. In comparison, 63,129 fry, or 86% of the second selection and 67% of the overall fry, were classified as low quality. The dominant fry type was Showa, with 8,587 fry (84% of the second selection and 9.2% of the overall fry), followed by Shiro, with 1,617 fry (16% of the second selection and 1.7% of the overall fry).

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