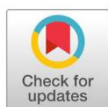


Broodstock maintenance technique of koi fish (*Cyprinus rubrofuscus*, Lacepede 1803) at the Center Fisheries of Freshwater Aquaculture, Sukabumi, West Java

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Abstract

Koi fish (*Cyprinus rubrofuscus*) is an ornamental fish that has a beautiful body shape and color so it has high economic value. Good maintenance of koi fish parents will produce superior koi fish seeds. The aim of this research is to find out the techniques for raising parent koi fish from preparation to spawning and to find out the problems faced and how to handle them. The methods used in this research are active participation, observation and interviews. The parameters observed include fecundity, FR, HR, and water quality (temperature, pH, and DO). The data obtained from the research was then analyzed descriptively. Based on research that has been carried out, the highest fecundity, FR and HR data obtained during 2 consecutive spawning were 60,480 eggs, 95.8% and 100%. Spawning was carried out semi-artificially using the GnRH hormone with a ratio of the parent's body weight male and female 1:1. The average water quality results measured include a temperature of 23.8 °C, pH 6.7, and DO 3.2 and are included in optimal conditions for rearing parent koi fish.

Keywords: Broodstock maintenance; koi fish; spawning

Introduction

Freshwater and marine ornamental fish represent a highly promising commodity, as they have gained significant popularity among both domestic and international markets in recent years. According to data from the Ministry of Marine Affairs and Fisheries, approximately 1,100 freshwater fish species are traded globally, with Indonesia contributing around 400 species¹. However, only about 90 of these species are actively cultivated by local communities². Among introduced freshwater ornamental fish, koi (*C. carpio*) remains a prominent choice in the international market due to its high economic value and relatively stable price dynamics. Commonly referred to as *nishikigoi*, koi are highly regarded by enthusiasts in Indonesia for their elegant body shapes and vibrant color patterns. Popular koi varieties



that consistently command favorable and stable prices in the global market include Kohaku, Taisho, Sanshoku, Showa, Shiro, Utsuri, Shusui, Asagi, Goromo, Goshiki, Bekko, Tancho, Kinginrin, and Kawarimono².

Since 2011, Indonesia has ranked fifth in the global ornamental fish export market, following the Czech Republic, Thailand, Japan, and Singapore, with total export revenues reaching \$5.24 million. The primary importing countries for Indonesian ornamental fish include the United States, Japan, Hong Kong, Australia, and the United Kingdom. Notably, Indonesia holds a competitive advantage in exporting ornamental fish to Malaysia and Australia, with lower opportunity costs compared to other exporting nations³. Producing high-quality koi fish requires effective aquaculture management to ensure superior offspring and fry³. Hatchery techniques are a crucial aspect of aquaculture, as they play a pivotal role in producing fry that determine the success of subsequent cultivation stages. However, the demand for high-quality koi fry has yet to be fully met due to limited production capacity. Therefore, a comprehensive understanding of proper koi broodstock management is essential to producing superior offspring⁴.

Material and methods

The maintenance of koi broodstock was conducted from June 19 to August 19, 2023, at the Freshwater Aquaculture Development Center (BBPBAT), located in Selabatu Village, Cikole District, Sukabumi City, West Java Province. The methodology employed in this activity included active participation in the broodstock maintenance process, encompassing preparation, spawning, and post-spawning stages. Observations were made through direct observation and interviews with BBPBAT Sukabumi personnel, complemented by additional information gathered from relevant scientific articles.

Result and Discussion

Koi broodstock maintenance techniques

The primary goal of broodstock maintenance is to select and condition the fish to ensure gonad maturation, making them ready for spawning and capable of producing high-quality offspring⁵. The koi broodstock at BBPBAT Sukabumi consists of imported koi from Japan, with the current broodstock being offspring of those original Japanese koi. The koi broodstock maintenance process at BBPBAT Sukabumi involves several stages, including pond preparation, broodstock selection, spawning, water quality management, and feed management, and pest and disease control.

Pond preparation

Two types of ponds are utilized for koi broodstock maintenance at BBPBAT Sukabumi: permanent ponds commonly referred to as showrooms, measuring 11 m × 7 m × 1.8 m with water depths of 1.2 m to 1.5 m, and semi-permanent ponds measuring 26 m × 16 m × 2 m with similar water depths. Pond preparation is the initial stage in the koi breeding process, which includes preparing broodstock maintenance ponds, spawning ponds, larval rearing ponds, and nursery ponds at various stages. At BBPBAT Sukabumi, broodstock pond preparation involves both outdoor and semi-indoor setups. The primary objective is to maintain the broodstock until gonad maturation, ensuring they are ready for spawning⁵. The pond preparation process includes pond drying, cleaning filtration compartments (for

semi-indoor ponds), filling the pond with water, installing filter materials in filtration tanks (for semi-indoor ponds), and finally, stocking the koi broodstock.

Broodstock feed management

Proper nutrition is a critical factor in successful koi breeding, as it significantly influences gonad maturation and the quality of eggs and sperm produced by the broodstock^{6,7}. At BBPBAT Sukabumi, the feed provided to koi broodstock is tailored to their specific needs and functions during the maintenance period. The primary feed used is floating pellets, which allow caretakers to visually monitor feed consumption and determine when the fish are satiated². This practice aligns with the Indonesian National Standard⁸ (SNI 7775:2022), which specifies that floating pellets are suitable for gonad maturation in broodstock (**Table 1**).

Table 1. The type of feed and nutritional content

No	Type of feed	Specification	Nutritional content (%)	Description
1	Feed A	2 mm (d), floating (t), and 10 kg (p)	35 (P), 3 (F), 3 (CF), 12 (A), 12 (MC), and 2 (Ca)	Given to prospective broodstock in outdoor ponds
2	Feed B	1-5 mm (d), floating (t), and 20 kg (p)	50 (P), 9 (F), 2 (CF), 15 (A), and 11 (MC)	Given to broodstock in outdoor ponds
3	Feed C	8 mm (d), floating (t), and 15 kg (p)	35 (P), 3 (F), 3 (CF), 12 (A), 12 (MC), and 2 (Ca)	Given to prospective broodstock in semi-indoor ponds

Specification: d (diameter), t (type), and p (packaging); nutritional content (%) = P (protein), F (fat), CF (crude fiber), A (ash), MC (moisture content), and Ca (calcium).





Six types of feed (labelled A, B, and C) are used during the maintenance of koi broodstock at BBPBAT Sukabumi. The types of feed and their nutritional compositions are detailed in **Table 1**. This diverse feeding strategy ensures the broodstock receive adequate nutrition to optimize their reproductive performance. Feed A and Feed B are provided to broodstock maintained in outdoor ponds, while Feed C is given to broodstock in semi-indoor ponds. According to the Indonesian National Standard (SNI 7775:2022)⁸, the protein content in the feed provided for gonad maturation should be at least 30%. Based on this standard, the types of feed used for koi broodstock maintenance at BBPBAT Sukabumi meet the required protein content, as each feed contains more than 30% protein. The feed is distributed at a single point where the broodstock gather, slightly towards the center of the pond.



Figure 1. The process of GnRH hormone injection in female koi broodstock: left = preparation for hormone injection, and right = administration of GnRH hormone behind the dorsal fin of the broodstock

Feeding during broodstock maintenance is carried out to satiety, with feed given twice a day. Feeding to satiety means providing feed until the fish are full⁹. Feeding times are at 08:00 WIB and 15:00 WIB. The amount of feed provided is adjusted based on water temperature; if the water temperature is low, the amount of feed is reduced accordingly. According to SNI 7775:2022, the feed dosage for gonad maturation in broodstock is 2%-3% of biomass per day, with a minimum of two feedings per day⁸.

Table 2. Differences between male and female koi broodstock

Sex	First Spawning	Second Spawning	Description
Male			Long and slim body, genitalia not prominent or pointed
Female			Short and round body, enlarged and soft abdomen, prominent and round genitalia

In koi broodstock maintenance, immunostimulants are also provided in the form of feed enrichment. This enrichment involves floating pellets mixed with vitamin C, tetracycline capsules, and progol as a binder. These ingredients are thoroughly mixed into the pellets (coating) and then air-dried. The coated feed must not be sun-dried, as exposure to direct sunlight may cause the loss of vitamin C activity in the feed. According to Yuda and Suena¹⁰, vitamin C is particularly sensitive to degradation because it is easily oxidized at high temperatures, and this process is accelerated by heat, light, alkalis, enzymes, oxidizers, as well as copper and iron catalysts. In line with Taukhid and Lusiastuti¹¹, the addition of vitamin C to commercial feed has been shown to significantly improve survival rates when fish are challenged with koi herpesvirus (KHV), suggesting that vitamin C enhances the fish's immune response to KHV infections.

Weed and disease managements

Pests that interfere with koi broodstock maintenance include mongooses (*Herpestes javanicus*), snails, rice crabs, and wild fish. The mongoose, resembling a civet with a brown to reddish body, is commonly found in forests, shrublands, rice fields, ponds, and gardens¹². To prevent koi from being preyed upon by mongooses, a sufficient distance must be maintained between the pond's water level and the surrounding bank. Snails, which lay eggs on the walls around the pond, can serve as hosts for parasitic diseases, such as trematodes. *Lymnaea* spp. snails are known intermediate hosts for the trematode *Fasciola* sp.¹³. Rice crabs are pests that can damage the embankments of koi broodstock ponds by digging holes, leading to leaks. To prevent this, tarpaulins are used to seal the embankments. The

presence of wild fish poses competition for both food and space, and they can also serve as hosts for diseases.

Table 3. Fecundity data from koi broodstock spawning

Spawning	Broodstock	Weight (g)		Wg (g)	N (eggs)	Ws (g)	F (eggs)	
		Before	After					
27 2023	June	1	1,200	1,235	-	213	1	52.185
		2	1,400	1,600	-			
		3	1,900	1,655	245			
		4	1,600	1,705	-			
27 2023	July	1	2,410	2,230	180	288	1	51.840
		2	2,150	1,940	210			60.480
		3	1,660	1,550	110			31.680

Common diseases affecting koi broodstock at BBPBAT Sukabumi include KHV, *Aeromonas* sp., and tumors. KHV, caused by a virus from the *Alloherpesviridae* family, can result in up to 80–95% mortality in koi within two weeks¹⁴. Clinical signs of KHV infection include excessive mucus production, damage to the gills, and hemorrhaging on the skin, fins, and internal organs. Preventive measures include the use of immunostimulant-enriched feed. Another bacterial disease affecting koi broodstock is caused by *Aeromonas* sp., a pathogen known for its rapid spread and high pathogenicity, leading to motile *aeromonas* septicemia (MAS). Clinical symptoms of MAS include red lesions or sores on the fish's body¹⁵. Tumors, a non-infectious disease typically caused by environmental conditions, nutrient deficiencies, genetic factors, poor aquaculture management, or exposure to toxic substances, are also commonly observed. Tumors are not transmissible to other koi as they are the result of genetic abnormalities. At BBPBAT Sukabumi, the management of sick koi fish is carried out through quarantine procedures, where sick fish are separated from healthy ones to receive treatment. Before to quarantine, a sterilized fiber tank is prepared, which is treated with calcium hypochlorite for disinfection? The tank is then filled with water mixed with 1 gram of Acriflavine, and aeration is installed to supply oxygen.

Broodstock selection

Broodstock selection at BBPBAT Sukabumi is divided into two stages: selection of candidate broodstock and selection of mature gonad broodstock. The selection of candidate broodstock aims to identify high-quality fish to produce superior offspring. Candidate broodstock is selected from the harvest during each cultivation phase, based on body shape and coloration. After selection, male and female candidates are kept separately. The selection of mature gonad broodstock involves assessing body shape, the absence of defects, prominent coloration, and gonadal maturity. This aligns with the work of Ritonga et al.¹⁶, who emphasized that broodstock selection before spawning should focus on factors such as color brightness, color patterns, gonadal maturity, and the health of the fish. According to Iskandar et al.², male koi broodstock should be at least 1 year old with a minimum weight of 1 kg per fish, while female koi broodstock should be at least 2 years old and weigh at least 2 kg/fish. The differences between male and female koi broodstock during the first and second spawning are presented in **Table 2**.

The selection of koi broodstock begins with capturing the fish along the edge of the pond, followed by the evaluation of their morphology. This is in accordance with the statement by Lembang and Rahman¹⁷, who reported that the selection process begins by gathering all the fish to the pond's edge, where the broodstock is selected based on the observation of specific body parts that indicate gonadal maturity and readiness for spawning. For male broodstock, readiness for spawning is indicated by a rough operculum when touched and the release of sperm when the abdomen is gently squeezed. For female broodstock, readiness is signified by a smooth operculum, the release of eggs upon abdominal stripping, a swollen abdomen, and a reddish coloration around the genital opening (Table 2).

Table 4. Data on the FR from koi fish spawning

Spawning Tank	Sample eggs	Fertilized eggs	FR (%)
Spawning on 27 June 2023			
Tank 1	-	-	-
Tank 2	-	-	-
Tank 3 (Sanke)	213	192	90.1%
Tank 4	-	-	-
Spawning on 27 Juny 2023			
Tank 1 (Kohaku)	1,000	920	92.0%
Tank 2 (Sanke)	1,000	940	94.0%
Tank 3 (Platinum)	1,000	958	95.8%
Tank 4 (Ogon)	1,000	947	94.7%

Spawning

Spawning, as a key activity in fish breeding, serves two primary purposes: to produce new generations from the broodstock and to generate fish seeds for further cultivation to meet human consumption needs¹⁶. There are three primary spawning techniques in aquaculture: natural, semi-artificial, and artificial. In semi-artificial spawning, broodstock is injected with ovulation-inducing hormones to accelerate gonadal maturation and prepare the fish for spawning. After hormone injection, the broodstock is left to spawn naturally over a period of time. Before spawning, pond preparation is essential to prevent eggs from being affected by pests or diseases during the process. Pond preparation includes cleaning the fiber tanks of algae and debris, filling the tanks with water from a dug well, and installing hapa nets made of trilin fabric to facilitate larval harvesting⁶. The use of fiber tanks is preferred as they are easier to clean compared to concrete tanks, thus reducing the risk of pest and disease outbreaks.

A body weight ratio of 1:1 is used for each spawning event. The female koi broodstock is weighed before and after spawning. The purpose of this weighing is to calculate the gonad weight of the broodstock and determine the appropriate dose of GnRH hormone for injection. This hormone accelerates the process of ovulation and spawning. The GnRH hormone is administered at a dose of 0.5 ml per 1 kg of broodstock. The injection is performed using a 1 ml syringe, with the needle inserted intramuscularly at a 45° angle, just behind the dorsal fin¹⁸. After the injection, the broodstock is placed in the spawning tank, which contains floating mats (*kakaban*) to serve as a substrate for egg attachment. The process of GnRH hormone injection in female koi broodstock is illustrated in Figure 1.

Egg hatching

After spawning, both the male and female broodstock are removed and returned to their respective maintenance ponds. The broodstock is collected in the morning after ovulation has reached its peak. The separation of koi broodstock from their eggs is necessary to prevent the fish from consuming their own eggs¹⁹. Koi eggs hatch within 2 to 3 days post-spawning (Table 5). The egg hatching process takes place in the spawning tank, where aeration is used to maintain stable dissolved oxygen (DO) levels. The following are data on fecundity, fertilization rate (FR), and hatching rate (HR) obtained from two spawning events during the study.

Table 5. HR data for koi broodstock spawning

Spawning Tank	Sample eggs	Fertilized eggs	Hatched eggs	HR (%)	Temperature (°C)
Spawning on 27 June 2023					
Tank 1	-	-	-	-	-
Tank 2	-	-	-	-	-
Tank 3 (Sanke)	213	192	192	100.0%	-
Tank 4	-	-	-	-	-
Spawning on 27 July 2023					
Tank 1 (Kohaku)	1,000	920	486	52.8%	23.2
Tank 2 (Sanke)	1,000	940	770	81.9%	23.0
Tank 3 (Platinum)	1,000	958	362	37.8%	24.0
Tank 4 (Ogon)	1,000	947	800	84.5%	23.5

Fecundity

Fecundity refers to the number of mature eggs released during spawning. According to Jayanti et al. (2021), fecundity calculation is used to predict the number of eggs produced by the broodstock. Fecundity is measured based on the weight of the broodstock before and after spawning (Kusrini et al., 2015). The fecundity obtained during the first spawning was 52,185 eggs, while the fecundity during the second spawning for the first, second, and third broodstock was 51,840, 60,480, and 31,680 eggs, respectively (Table 3). According to Nurhayati et al.⁴, two factors influence fecundity: internal factors such as the fish species or genetics, and external factors such as temperature, feed, and environmental conditions. Fecundity is also affected by the protein content in the feed. The higher the protein content in the feed, the higher the fecundity²⁰. Based on these findings, it can be inferred that the high fecundity of the female broodstock maintained in outdoor ponds is due to the higher protein content in the feed compared to the feed used in indoor ponds.

Fertilization rate (FR)

Fertilization rate, or the degree of fertilization, refers to the number of fertilized eggs relative to the total number of eggs. The definition of FR is the percentage of eggs that are fertilized out of the total number of eggs laid during spawning²¹. FR represents the percentage of fertilized eggs from the total eggs produced in one reproductive cycle or spawning. The calculation of the fertilization rate FR in the first spawning involved sampling a small portion of eggs attached to the *kakaban* (Table 4). These samples were not analyzed meticulously and were directly equated with the fecundity sample count. To

determine the number of fertilized eggs, unfertilized eggs were identified and subtracted. According to Ihwan et al.²⁰, fertilized eggs are translucent, whereas unfertilized eggs are milky white.

Table 6. Water quality measurements in koi broodstock maintenance ponds

Parameter	Pond				Spawning tank
	Semi-indoor		Outdoor		
	Male Broodstock	Female Broodstock	Male Broodstock	Female Broodstock	
Temperature (°C)	23.6	23.4	25.25	25.25	23.7
pH	6.81	6.59	6.65	8.42	6.91
DO (mg/L)	3.07	3.33	2.12	3.01	4.58

Hatching rate (HR)

The HR is determined by comparing the number of hatched eggs to the number of fertilized eggs. This parameter is observed after hatching⁵. The HR calculation was performed by sampling eggs using a shaking sieve containing 1,000 eggs per hatching tank. Observations were conducted one day after hatching (**Table 5**). The low hatching rate was attributed to the high number of unfertilized eggs, resulting from sperm failing to enter the egg micropyle and excessive egg density, which caused clumping and decomposition. Additionally, some eggs failed to develop post-fertilization due to physiological disruptions during embryogenesis²⁰.

The temperature in the hatching tanks during the second spawning ranged from 23.0-24.0 °C (23.2, 23.0, 24.0, and 23.5 °C). Higher temperatures generally accelerate hatching²². However, extreme or abrupt temperature changes can inhibit hatching and lead to embryo mortality. According to Darnalim and Ikhlas²², the optimal temperature range for egg hatching is 24–31°C. This range supports embryonic development and metabolic processes essential for organogenesis within the eggs (**Table 5**).

Water quality

Monitoring water quality parameters is essential to assess the current condition of the aquatic environment, serving as a reference for taking necessary actions to maintain and sustain optimal water quality for koi survival. During this study, water quality assessments were conducted twice at the Water Quality Laboratory of BBPBAT Sukabumi. Water sampling was performed by directly collecting water from koi maintenance ponds and transferring it into sample bottles. The parameters tested at BBPBAT Sukabumi included temperature, pH, and DO. The results of the water quality observations in the BBPBAT Sukabumi koi ponds are presented in **Table 6**.

According to Sa'adati and Andayani²³, the ideal temperature range for koi cultivation is between 25–28°C, while the Indonesian National Standard (SNI 7775:2022) specifies an optimal range of 20–30°C for koi maintenance media⁸. Temperature is a critical factor in aquaculture activities. As shown in **Table 6**, the water temperature in the koi broodstock maintenance ponds falls within the optimal range. Fish metabolism is directly proportional to water temperature; higher temperatures increase metabolic activity, and lower temperatures decrease it²⁴. The acidity level (pH) is another significant water quality parameter that greatly influences the survival of koi. According to Nurhayati et al.⁴, the optimal pH range for koi growth is 6.5–8.5. The recorded pH values are within the optimal range for koi survival, ranging from 6.59 to 8.42.

DO is essential for the respiration of cultured organisms, growth metabolism, and the aerobic oxidation of organic and inorganic waste¹⁷. The DO values observed in the koi broodstock maintenance ponds were suboptimal for supporting koi survival, ranging from 2.12 to 3.33 mg/L (**Table 6**). According to Bayu and Fasya⁷, the normal DO range for maintaining koi broodstock is 5.85–7.68 mg/L. Additionally, the Indonesian National Standard (SNI 7734:2017) specifies that the minimum optimal DO level for broodstock maintenance is 5 mg/L⁸. Simamora et al.²⁵ noted that koi can survive at DO levels below 5 ppm; however, such low oxygen levels can result in reduced appetite and stress.

Conclusions

The highest values of fecundity, fertilization rate (FR), and hatching rate (HR) achieved during the two spawning cycles were 60,480 eggs, 95.8%, and 100%, respectively. The best spawning results were obtained from broodstock fed a diet with high protein content. Furthermore, water quality parameters observed during the study were generally within the optimal range for koi broodstock maintenance. The maintenance process adhered to the standards outlined in the Indonesian National Standard (SNI).

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Conflicts of Interest

The authors declare no conflict of interest

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