Enhancing Reproductive Performance of Nilem Fish (*Osteochilus hasselti*) through Artificial Spawning

Abstract

This study aims to evaluate the reproductive performance of Nilem fish (*Osteochilus hasselti*) through artificial spawning methods. Spawning was conducted in glass aquaria using the sGnRH-a hormone on both male and female broodstocks. Egg production, fertilization rate (FR), hatching rate (HR), and survival rate (SR) were the main observed parameters. The research results revealed that the average egg production per female broodstock reached 25,350, indicating a high level of fecundity. The fertilization rate reached 97.70%, indicating an effective egg fertilization process. The hatching rate (HR) value of 92.20% indicates success in the egg-hatching process. Larval survival rate (SR) during the 4-day rearing period reached 84.70%. Maintaining optimal water quality also contributed to the success of artificial spawning. In conclusion, artificial spawning in Nilem fish could achieve high levels of success by optimizing reproductive parameters and considering environmental factors. This study provides valuable insights into the sustainable development of Nilem fish cultivation.

Keywords: Nilem fish, artificial spawning, reproductive performance, egg production, fertilization rate, hatching rate, survival rate.

1. INTRODUCTION

Nilem fish (*Osteochilus hasselti*) is one of the potential freshwater fish commodities, yet it has not been widely cultivated [1]. The excellence of Nilem fish lies in its role as a bio-cleaning agent, as it tends to consume detritus, periphyton, and algae [2]. Its distribution areas in South Asia include Sumatra, Java, Kalimantan, Sulawesi, Malaysia, Thailand, Singapore, Vietnam, and Cambodia [2]. The Nilem fish has a unique and delicious flavor compared to other freshwater fish. This is because the meat contains glutamic acid, naturally occurring in the fish. This flavor might be influenced by the fish's diet of plankton, which is algae that grow due to pond fertilization [3].

The market prospects for Nilem fish are quite broad, not only in the local market but also reaching the international market. Nilem fish cultivation is considered beneficial from health, economic, and environmental sustainability perspectives. Nilem fish is classified as omnivorous [3,4,5].

The presence of Nilem fish populations in public waters is decreasing. The decline in the fish population is not only due to exploitation but is also suspected to be a result of changes in the aquatic environment. One step that can be taken to increase production and ensure the sustainability of Nilem fish is through the development of Nilem fish farming activities in the community. Therefore, the production and provision of fish seeds can be enhanced, both as a commercial commodity and for

the improvement of fish populations in public waters through restocking activities [5]. In efforts to develop Nilem fish production, research on the reproductive performance of these fish is required. This study aims to determine the reproductive performance of Nilem fish (*O. hasselti*) bred artificially.

2. MATERIALS AND METHODS

This study employed an exploratory method with seven replications. The research was conducted in the field laboratory of the Technical Implementation Unit of Freshwater Fish Cultivation Department (LTIU-FFCD), Kutasari, Purbalingga, Indonesia from January to March 2023.

2.1. Broodstock and Its Maintenance

The test fish consisted of 28 males of Nilem (*Osteochilus hasselti*) broodstock with an average weight of 111.5±5.75 g and 21 female broodstock with an average weight of 220.00±14.14 g, sourced from LTIU-FFCD, Purbalingga. The test fish were selected based on gonadal maturity, physical characteristics, and age of the broodstock. Male Nilem fish have slender bodies, agile movements, and release a thick white substance when stripped from the abdominal region. Female broodstock exhibits a stout body posture, a relatively enlarged and soft abdomen, slow movements, a slightly dilated round egg genital opening, and the release of clear yellowish fluid from the genital opening.

The test fish (broodstock) were raised in a 35 m^2 broodstock pond, with dimensions of 7 m in length and 5 m in width. The water depth in the broodstock pond was 80 cm. Freshwater from the river stream was used in the broodstock maintenance pond. The test broodstocks were fed on pellets containing 33% of protein, 5% of fat, 6% of fiber, 13% of ash, and 13% of water. Feeding was carried out twice a day using the 'at satiation method', and additional feed in the form of water spinach leaves was provided.

2.2. Breeding of Test Fish

The breeding of the test fish broodstock was carried out by injecting the synthetic hormone sGnRH-a [6]. The hormone dosage used for injecting male Nilem fish was 0.4 mL/kg body weight, while for female broodstock, it was 0.5 mL/kg. The injection was performed on the dorsal part towards the intramuscular area at a 45-degree angle. The spawning process took place in 7 glass aquaria with dimensions of (80x5x30) cm^3, where the water height was maintained at 20 cm, and the aquaria were equipped with aeration. Semi-artificial breeding was conducted with a ratio of 4 males to 3 females in each replication. The Nilem fish (*O. hasselti*) breeding procedure is outlined in Table 1.

Table 1. Nilem Fish (*Osteochilus hasselti*) Spawning Procedure

No.	Local Time	Description
1.	16.00	Injection of male and female broodstock with hormones
2.	17.00	Introduction process of male and female

		broodstock
3.	00.00	Male and female broodstock engaged in chasing behavior
4.	01.00	The first spawning process occurred
5.	02.00	Transferred eggs to the hatching aquarium
6.	03.00	Spawning process completed

2.3. Hatching of Eggs and Larval Rearing

Egg hatching was conducted in 21 aquaria measuring (80x50x30) cm^3 with a water height of 20 cm. During the hatching process, oxygen requirements were supplied using an aerator. The eggs hatched approximately 24 hours after being released from the broodstock. Subsequently, the larvae were reared for 4 days, and water quality was maintained within optimal limits.

2.4. Observed Variables

Egg Production

The number of eggs was determined by counting the total number of eggs produced by the female broodstocks. The counting of eggs was done through sampling using a 20 mL measuring glass. The result of counting the eggs in 20 mL was then multiplied by the total number of measuring glasses.

Fertilization Rate (FR)

Fertilization rate (FR) is the degree of egg fertilization carried out by the male broodstock. The FR value depends on the quality of the eggs and the sperm [7]. The FR value was calculated using the formula:

$$\text{FR}\ (\%) = \frac{\sum \text{fertilized eggs}}{\sum \text{released eggs}}\ \text{x}\ 100$$

Hatching Rate (HR)

The Hatching Rate (HR) is a parameter used to assess the degree of egg hatching [7]. The HR value was calculated by using the formula:

$$\text{HR}\ (\%) = \frac{\sum \text{hatched eggs}}{\sum \text{fertilized eggs}}\ \text{x}\ 100$$

Survival Rate (SR)

Survival rate (SR) is the comparison of the number of organisms alive at the end of a period to the number of organisms alive at the beginning of the period. Survival rate can be used as a parameter to assess the tolerance and ability of fish to survive [8]. The SR value was calculated by using the formula:

$$\text{SR}\ (\%) = \frac{\sum \text{number of larvae at the end of the rearing period}}{\sum\ \text{number of larvae at the initial of the rearing period}}\ \text{x}\ 100$$

2.5. Data Analysis

The obtained data from the research results were further subjected to descriptive statistical analysis.

3. RESULTS AND DISCUSSION

3.1. Results

The reproductive performance of Nilem fish (*Osteochilus hasselti*), including egg production, fertilization rate (FR), hatching rate (HR), and survival rate (SR) are presented in Table 2. Spawning was conducted in glass aquaria using semi-artificial methods with sGnRH-a hormone at a dosage of 0.4 mL/kg for male broodstock and 0.5 mL/kg for female broodstock. The female Nilem fish (*O. hasselti*) exhibited an average egg production capacity of 25,350±110 eggs, with an average weight of female broodstock at 220.00±14.14 g.

Table 2. The Reproductive Performance of Nilem Fish (*Osteochilus hasselti*) Induced by sGnRH-a Hormone Injection

Reproduction parameters	Value
Egg production (eggs)	25,350 ± 110
Fertilization rate (FR, %)	97.70 ± 0.96
Hatching rate (HR, %)	92.20 ± 1.18
Larval production (larva)	22,835.40 ± 855.73
Survival rate (SR, %)	84.70 ± 0.74

Measurement of water quality parameters was conducted in the spawning aquarium, broodstock maintenance pond, and egg-hatching aquarium. The results of the water quality parameter measurements, including pH, temperature, and dissolved oxygen (DO) during the spawning process are presented in Table 3.

Table 3. Results of Water Quality Measurements during the Spawning Process of Nilem Fish (*Osteochilus hasselti*)

Time	Container								
	Broodstock pond			Spawning aquarium			Hatching eggs and rearing larvae aquarium		
	pH	DO (mg/L)	Temperature (^{0}C)	pH	DO (mg/L)	Temperature (^{0}C)	pH	DO (mg/L)	Temperature (^{0}C)
Morning (08.00)	7.18± 0.36	8.62± 0.43	26.0± 1.44	7.6± 0.00	5.40± 0.00	27.30± 0.00	7.65± 0.04	7.35± 0.99	27.18± 0.31
Afternoon (14.00)	7.62± 0.35	6.13± 0.86	27.63± 0.71	7.5± 0.00	6.60± 0.00	27.60± 0.00	7.68± 0.17	7.51± 0.83	27.28± 0.68

3.2. Discussion

The number of eggs produced by a female Nilem broodstock reaches 25,350±110 eggs. This high fecundity is consistent with the reproductive characteristics of Nilem, as described by Rosyida et al. [6] and Hadiroseyani et al. [9], stating that Nilem fish have high reproductive potential and fecundity. For comparison, a pair of Nilem weighing between 100-150 g can produce between 15,000 to 30,000 eggs per individual. Similar statements were also made by Subagja et al. [10] and Yuniarti et al. [11], indicating that the fecundity of Nilem reaches 18 to 20% of the body weight of the female broodstock with a weight of 150 g, equivalent to 20,000 to 40,000 eggs. If used as seeds, the number of seeds produced ranges from 15,000 to 30,000 individuals per breeding pair.

The number of eggs produced is influenced by the level of gonadal maturity and the condition of the female broodstock, as observed from the several spawning events that the broodstock has undergone. The Nilem fish broodstock used for spawning is sourced from the hatchery itself. According to Mujadid et al. [12], the number of eggs produced by a female is related to its size. The number of eggs will increase with the growth of the broodstock and the level of gonadal maturity.

Fertilization is the process of the sperm nucleus merging with the egg cell nucleus in the cytoplasm, forming a zygote. The observed fertilization rate (FR) in Nilem fish spawning is 97.70±0.96%. The number of unfertilized eggs is 576±30 eggs. Fertilized eggs have transparent characteristics, while unfertilized eggs appear opaque white. This observation aligns with the statement made by Putri et al. [13] and Rosyida et al. [6] that fertilized eggs will be transparent, and if they appear milky white, it indicates that the eggs are not fertilized and should be separated promptly.

The degree of fertilization in Nilem fish was influenced by environmental conditions, egg quality, and sperm quality [14,15]. The success of fertilization in fish was highly influenced by external and internal factors. External factors included fertilization and hatching media, while internal factors included the viability and quality of eggs and sperm [6,14,16]. The degree of fertilization in fish was greatly determined by the quality of eggs, spermatozoa, media, and human handling [17]. Eggs placed in water will quickly swell, expediting the microphile closure process. The time required by spermatozoa to fertilize the egg cell was very short.

The results obtained indicated a successful egg-hatching process, with a total of 22,835.40±855.73 larvae produced. The number of non-hatching eggs was 1,930±47 eggs. The hatching rate (HR) was recorded at 92.20±1.18%. The egg-hatching process occurred 12 hours after the eggs were released from the broodstock. The high hatching rate was attributed to the good quality of the eggs, as evidenced by their transparent and large characteristics. This observation was supported by Rosyida et al. [6] and Firmatin et al. [18], who stated that the internal factor influencing the low egg hatching success was the quality and diameter of ovulated eggs. While the eggs were successfully fertilized by spermatozoa, the embryos may not develop properly due to poor egg quality.

The difference in hatching rates can be attributed to varying egg contents or compositions in each species, resulting in different responses to male fish sperm [6]. The egg-hatching process and activities within the egg were more influenced by

environmental conditions, especially temperature [13]. Additionally, it was affected by factors such as pH, carbon dioxide levels, light intensity, and oxygen absorption.

The result of larval survival rate (SR) during the 4-day rearing period was 84.70±0.74%. However, the number of dead larvae was relatively high, totaling 5,990±32.00 individuals. Larvae from days 1 to 4 were reared without being fed, as they still have a yolk sac. The larval phase was a critical stage in the breeding of Nilem fish because larvae were highly sensitive to environmental factors. During the larval phase, fish were not yet capable of adapting to the environment [19]. Additionally, another factor contributing to high larval mortality was the difficulty of the larvae to obtain suitable food due to the smaller size of their mouth opening [14].

Survival rate is the percentage of fish that survive during the rearing period. Factors influencing the high or low survival rate of Nilem fish during larval rearing included environmental factors [20]. The low survival rate was attributed to environmental factors, density, and stress due to media and water changes [21]. A similar statement was echoed by Astria et al. [22], stating that fish survival is determined by the availability of food, the quantity of food, health, and the cultivation environment.

Another factor influencing the high or low survival rate of Nilem fish larvae, besides external factors, was the availability of food reserves in the fish. Larvae that have not received external food and still rely on the yolk sac as their main source of energy will be influenced in terms of survival [18]. The energy source for the early development of fish larvae, after the eggs hatch, heavily depended on the inherent egg material prepared by the broodstock. The yolk sac serves as the primary source of nutrition and energy for the larvae during the endogenous feeding process, which begins at fertilization and ends when the larvae start obtaining external food.

Water quality is a crucial factor in fish farming. Key water quality parameters that play a significant role in fish spawning include temperature, pH, and dissolved oxygen (DO). Based on the observed water quality in the broodstock maintenance pond, the temperature ranged from 25.8 to 28.6ºC, pH from 6.9 to 7.68, and DO from 8.1 to 9.1 mg/L. In the spawning aquarium, the recorded values were temperature 27.3ºC, pH 7.64, and DO 5.4 mg/L. Meanwhile, in the egg-hatching aquarium, the temperature ranged from 26.9 to 27.6ºC, pH from 7.6 to 7.69, and DO from 6.4 to 8.7 mg/L. The observed water quality meets the necessary criteria and remains within the optimal range for the survival of Nilem fish. Freshwater fish such as gourami, Nilem, and carp generally thrive at temperatures between 26 and 30ºC, with optimal pH levels between 6 and 8 and optimum DO for Nilem fish ranging between 5 and 8 mg/L [23,24]. Water quality in aquaculture is a factor that influences the survival, reproduction, growth, and production of fish [25,26]. An optimal temperature for fish life in tropical areas ranges between 25 and 30ºC. Several studies indicated that Nilem fish can thrive well at temperatures ranging between 18 and 28ºC [9,27,28].

4. CONCLUSION

Artificial spawning in Nilem fish (*Osteochilus hasselti*) can achieve a significant level of success, evident from adequate egg production and hatching. Water quality, particularly temperature, oxygen, and pH, played a crucial role in supporting reproductive success. The health and maintenance conditions of the broodstock

directly impacted egg production and larval quality. Sufficient food availability during spawning was a key factor in supporting the well-being and reproduction of Nilem fish. Despite the success, there were challenges such as the survival rate of larvae that needed attention. Further development may involve improving the management of maintenance and spawning to support larger and more sustainable production.

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