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Improving the Hatching Rate of Nilem Fish (Osteochilus hasselti) Eggs through the Addition of Thyroxine to the Hatching Medium

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A B S T R A C T

The Nilem fish (Osteochilus hasselti) is an indigenous fish of Indonesia with significant potential and strategic value for development into a leading freshwater fish commodity. With a low egg hatching rate of 60%, there is a need for research to improve the hatching success of Nilem fish eggs. One method involves using media containing thyroxine during the hatching process. Thyroxine stimulates the thyroid gland, which is crucial for accelerating metamorphosis and promoting the development and growth of fish, particularly during the critical embryo and larval stages. It is anticipated that soaking eggs in thyroxine at specific doses can enhance the hatching rate of Nilem fish eggs. This study aims to assess the impact of thyroxine on the hatching rate and hatching time of the eggs. The research was conducted at the Freshwater Fish Hatchery and Cultivation Unit (FFHCU) in Ngrajek, Magelang, Central Java, Indonesia, using Nilem fish eggs (Osteochilus hasselti) for testing. An experimental method was employed with a completely randomized design (CRD), featuring 2 treatments and 3 replications. The thyroxine treatment doses were A (0 mg/L) and B (0.15 mg/L). The thyroxine, in powdered form, was ground using a mortar to facilitate the soaking process. Observed data included egg hatching rate, hatching time, and water quality. Results indicated that the hatching rate was 61.61 ± 2.54% for treatment A and 79.73 ± 2.44% for treatment B. The hatching time was consistent across treatments at 27.00 ± 0.10 hours. Water quality parameters, including temperature, dissolved oxygen, and pH, were within suitable ranges for hatching Nilem fish eggs: temperature ranged from 27.70-29.40˚C, dissolved oxygen from 5.10-6.30 mg/L, and pH from 6.00-6.90.

Keywords: Osteochilus hasselti, hatching water medium, thyroxine, Nilem fish eggs, hatching rate

1. Introduction

The Nilem fish (Osteochilus hasselti) has potential and strategic value for development into a leading freshwater fish commodity. The demand for Nilem fish continues to increase year by year. However, the current production of Nilem fish is limited to 385,701,378 kg per year. This low production value is due to limited efforts and because Nilem fish farming is still a side activity (Nur & Sriati, 2015; Hastuti et al., 2024a). One of the factors determining the success of aquaculture production is the availability of good quality seeds. High-quality seeds are characterized by being healthy, without physical defects, agile in movement, and having bright body colors. Nilem fish farming is still challenging to control, resulting in decreased production quality. The hatching rate (HR) of Nilem fish is 32.43% (Susanto, 2006), which is considered low. Environmental changes in the Nilem fish habitat lead to high mortality rates during the embryo and larval phases. High mortality in these phases is due to the embryos' inability to develop and metabolize to form tissues for future organs (Hardaningsih & Rochmawati, 2008; Vebiola et al., 2019). It is noted that the embryo and larval stages are the most critical. Common issues in the embryo phase include incomplete embryogenesis and organogenesis, unequal metamorphosis, and slow growth. The rate of yolk absorption and fish growth can be increased through hormonal manipulation using thyroxine (Yuniarti et al., 2023).

The Nilem fish, Osteochilus hasselti, is native to Indonesia and lives in freshwater environments such as rivers and swamps (Syamsuri et al., 2017; Hastuti et al., 2024a). The production of Nilem fish is still relatively low, at 32,854.65 tons in 2021 (KKP, 2021). Hatchery seed production only reaches 40% (Hastuti et al., 2024a). Efforts to increase Nilem fish seed production include hormonal engineering. Hormonal engineering is done internally using thyroxine, which benefits larval or seed growth by soaking eggs (Vebiola et al., 2019; Yuniarti et al., 2023). Larvae soaked in thyroxine show higher growth rates due to increased metabolism (Muttaqin, 2012; Yuniarti et al., 2023).

Thyroxine is a hormone that plays a role in promoting fish growth (Yuniarti et al., 2023; Pratama et al., 2022). Produced by the thyroid gland, thyroxine contains iodine, which is essential for growing tissues, such as muscle and heart cells in larvae. Thyroxine is believed to enhance nerve and muscle tissue functions, making them more active and supporting seed or larval growth (Oktaviani et al., 2017). It can stimulate the oxidation rate of food materials, increase growth, boost oxygen consumption, and accelerate metamorphosis (Khalil et al., 2011). According to Tripathi and Verma (2003), factors influencing thyroxine activity include concentration (dose), method of administration, feed quality, feeding time, and fish size. Various studies on thyroxine administration in fish have shown inconsistent responses. These variations are due to differences in dosing methods, hormone types, doses, and treatment durations. Kurniawan et al. (2014) stated that fish stage affects sensitivity to thyroxine.

So far, thyroxine hormone use in Nilem fish has only been applied at the seed stage with varying doses in both feed and media (Hastuti et al., 2024a; Yuniarti et al., 2023). Research to optimize thyroxine hormone use in enhancing Nilem fish embryo growth during egg hatching is crucial. A study on the application of thyroxine through water in the Nilem fish egg hatching medium is needed to improve the hatching rate. Therefore, this study aims to increase the hatching rate of Nilem fish eggs (*Osteochilus hasselti*) by adding thyroxine to the hatching medium.

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2. Materials and Methods

2.1. Test Materials

This study used Nilem fish eggs (Osteochilus hasselti) sourced from broodstock spawning at the Freshwater Fish Hatchery and Cultivation Unit (FFHCU) in Ngrajek, Magelang, Central Java, Indonesia. Fertilized eggs were selected for testing. A total of 250 test eggs were hatched in aquarium containers, with a total of 1500 eggs used. The thyroxine used was Thyrax, with an active ingredient of 0.1 mg per tablet.

2.2. Hatching Procedure

The test eggs were hatched in glass aquariums measuring 30 x 30 x 50 cm. Six aquariums equipped with aeration were used as experimental containers. Each aquarium was filled with 3 liters of water. A thyroxine concentration of 0.15 mg/L was achieved by crushing 4.5 Thyrax tablets. The thyroxine was then added to the 3 liters of hatching media water. The test fish eggs were maintained in the aquariums until they hatched.

2.3. Experimental Design

A completely randomized design (CRD) was applied, with 2 treatments and 3 replications. The treatments were as follows:

- A: Hatching Nilem fish eggs in media without thyroxine (0 mg/L)
- B: Hatching Nilem fish eggs in media containing 0.15 mg/L thyroxine

2.4. Research Variables

The data collected included the egg hatching rate, hatching time, and water quality parameters. Water quality parameters measured were temperature, dissolved oxygen (DO), and pH.

2.4.1. Hatching Rate (HR)

The percentage of eggs hatched in each treatment was calculated using the formula proposed by Effendie (2002).

The formula to calculate the hatching rate (HR) used is:

$$\text{Hatching Rate (HR)} = \frac{\text{Number of eggs hatched}}{\text{Total number of eggs}} \times 100\% \qquad (1)$$

2.4.2. Hatching Time

Hatching time is the duration (in hours) taken for the eggs to hatch into larvae.

2.4.3. Water Quality

Water quality parameters measured included temperature (°C), pH, and dissolved oxygen (mg/L). Measurements were taken from the time the eggs were introduced until they hatched.

2.5. Statistic Analysis

The experimental data analysis was conducted to identify differences in response to the treatments. The hatching rate and hatching time were assessed using a Student's t-test. Meanwhile, water quality parameters consisting of temperature, dissolved oxygen and pH, is analyzed descriptively.

3. Results and Discussion

3.1 Results

The hatching time and hatching rate of Nilem fish (*Osteochilus hasselti*) eggs reared in media containing thyroxine are presented in Table 1. An illustration of the effect of thyroxine on the hatching rate of eggs is shown in Figure 1. Water quality parameters were also presented in Table 1. The relationship between thyroxine concentration in the hatching media and changes in the hatching rate of Nilem fish eggs is described by the regression equation Y = 120.77X + 61.613, with an R^2 value was 0.9635.

Table 1. Hatching time and hatching rate of nilem fish (*Osteochilus hasselti*) eggs reared in water containing thyroxine

Variable	**Thyroxine concentration in hatching water media (mg/L)**	
	0	**0.15**
Hatching time (hours)	27.00±0.10[a]	27.00±0.10[a]
Hatching rate (%)	61.61±2.54[a]	79.73±2.44[b]
Water quality parameters		
Temperature (ºC)	27.70-29.40	27.70-29.00
Dissolved oxygen (mg/L)	5.10-6.30	5.20-6.40
pH	6.00-6.90	6.00-6.85

Figure 1. Graph of the regression equation for the hatching rate response of nilem fish eggs reared in water containing thyroxine

3.2 Discussion

Embryo development for each treatment from the first to the second hour showed cleavage phase progression. By the third hour, embryos reached the morula stage. However, at the fourth hour, different treatments showed varied progress: Treatment A (0 mg/L thyroxine) remained in the blastula phase, while Treatment B (0.15 mg/L thyroxine) advanced to the early gastrula phase. By the fifth hour, both treatments were in the gastrula phase. By the seventh hour, Treatment A was still in the gastrula phase, whereas Treatment B had progressed to early organogenesis, marked by the formation of the vertebral column. At the nineteenth hour, organogenesis included the formation of the brain and heart. Embryos hatched at the twenty-seventh hour in both Treatment A and Treatment B.

According to Hutagalung et al. (2017), during the blastodis phase, cells divide into 2, 4, 6, 8, 16, and 32 cells within 55 minutes. After reaching 32 cells, the morula phase begins at the third hour, followed by the blastula phase between the third and fourth hours, early gastrula phase between the fourth and sixth hours, and organogenesis phase between the eighth and tenth hours. Organogenesis is critical for forming various fish organs, such as the vertebral column, eye cavities, gills, heart, and fins.

The study results indicated that adding thyroxine (0.15 mg/L) to the hatching media did not significantly accelerate the hatching time of Nilem fish eggs, which was 27.00 ± 0.10 hours (Table 1). The thyroxine concentration did not adversely affect the embryo development of Nilem fish, as thyroxine contains iodine that enhances metabolism and protein breakdown, providing energy for embryo and larval stages. According to Muslim et al. (2019), thyroxine enhances protein utilization and metabolism, which is essential for sustaining life through biochemical reactions. Faster metabolism leads to increased energy production for cell growth. Vebiola et al. (2019) suggested that thyroxine diffuses osmotically into the eggs, increasing RNA and mRNA synthesis, which accelerates protein synthesis. Protein is crucial for fish growth, thus enhancing the growth and development of embryos and larvae.

The hatching rate of Nilem fish eggs in 0.15 mg/L thyroxine media was 79.73 ± 2.44%, significantly higher than the 61.61 ± 2.54% in the control group (Table 1). A similar pattern was observed in Tawes fish eggs, with a 58.83% hatching rate without thyroxine (Harahap, 2018). The increased hatching percentage was attributed to mechanical processes within the eggs, such as the stronger embryo position due to iodine from thyroxine, leading to larger embryos breaking the eggshell. Chorionase enzyme activity, which weakens the chorion membrane, also facilitates hatching. According to Altiara et al. (2016), chorionase, a protease enzyme produced by hatching gland cells, plays a critical role in hatching. When fish eggs contact water, the chorion membrane detaches, creating a perivitelline space that cushions the embryo during development (Manurung et al., 2017).

Thyroxine's effect on hatching rate is illustrated in Figure 1, with a regression equation Y = 120.77X + 61.613 and R2 = 0.9635. This shows a 96.35% correlation between thyroxine concentration and hatching rate, predicting a 120.77% increase in hatching rate per 1 mg/L thyroxine. Thus, a 0.15 mg/L thyroxine concentration would increase the hatching rate by 18.1155%.

Thyroxine likely enters the egg via osmosis, influenced by osmotic pressure differences and protein imbibition on the egg yolk surface, aiding its absorption. The vitelline membrane prevents water entry into the embryo, with the expanding perivitelline cavity allowing free embryo movement, leading to hatching (Rahman, 2016). Chorion weakening by chorionase facilitates hatching.

The hatching rates of 61.61 ± 2.54% (Treatment A) and 79.73 ± 2.44% (Treatment B) indicate hatching failures due to factors like substrate, hatching media, or water quality. According to Hardaningsih & Rochmawati (2018), embryo development failures can result from insufficient metabolic processes, affecting tissue formation. Thyroxine's iodine content serves as an antifungal agent, preventing embryo mortality. Thus, thyroxine-treated media showed higher hatching rates (Nayak et al., 2004).

Successful Nilem fish egg hatching depends on environmental factors, hatching containers, and media. Aeration ensures oxygen distribution in hatching media, with dissolved oxygen levels between 5.10-6.40 mg/L deemed adequate (Syamsyuri et al., 2017). Optimal dissolved oxygen ranges from 5.00-7.00 mg/L (Syamsyuri et al., 2017). Suitable temperature and pH values are 25-30°C (SNI, 1999) and 6.00-9.00 pH (Ulyana et al., 2018). Proper water quality is vital for fish embryo development, as oxygen supports growth, and insufficient oxygen leads to larval mortality (Herawati et al., 2007). Aeration increases oxygen levels in hatching media. Hatching water temperature ranged from 27.70-29.40°C, remaining constant. Higher temperatures accelerate hatching by increasing embryo activity. Prakoso and Kurniawan (2015) and Hastuti et al. (2024b) noted that temperature affects chorionase activity and embryo development. Fish eggs are sensitive to temperature fluctuations, with high temperatures hindering tissue formation and causing abnormalities or mortality (Landsman et al., 2011). Besides dissolved oxygen and temperature, pH affects Nilem fish egg hatching (Hastuti et al., 2024a). The optimal pH for Nilem fish egg hatching is 6.00-6.90 (SNI, 1999). Optimal pH for larval rearing is 6.5-8.5 (SNI, 1999), while Nilem fish develop at pH 8.00-9.00, with an optimal embryonic development range of 6.00-9.00 (Ulyana et al., 2018). Water quality parameters for Nilem fish egg hatching media fall within the optimal range for embryo development.

4. Conclusion

Thyroxine at a concentration of 0.15 mg/L in hatching media water increases the hatching rate of Nilem fish (Osteochilus hasselti) eggs but does not accelerate the hatching time. The hatching rate reached 79.73 ± 2.44%, with a hatching time of 27.00 ± 0.10 hours. The increase in the egg hatching rate follows the regression equation Y = 120.77X + 61.613, with an R2 value is 0.9635. Nilem fish eggs hatched in media water containing 0.15 mg/L thyroxine showed an increase in the egg hatching rate by 18.1155%.Acknowledgements

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