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The effect of temperature on the hatchability of eggs soaked in tannin and the survival of goldfish (*Carassius auratus*)

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Abstract
The issue in goldfish egg production is the low hatching rate and the high adhesiveness of the eggs. The aim of this research was to optimization the temperature for incubating goldfish eggs that have been pre-immersion in a tannin solution (6 g/L). The research method used was an experimental method with 4 treatments: A (24 °C), B (26 °C), C (28 °C), D (30 °C). The results indicate that both temperature and tannin pre-treatment significantly affect the hatching rate and survival of goldfish. The best treatments were C and D, with fertilization rate of 94.33% and 97.00%, hatching rate were 90.33% and 94.33%, and survival rate were 83.67% and 88.33%, respectively. The least time for egg hatching, which was 3,120 minutes. It is suggested to farmers can use an incubation temperature of 28 °C to achieve good hatching rates and survival while saving production costs.

Keywords: Goldfish, hatching rate, survival rate, tannin

1. Introduction
The goldfish (*Carassius auratus*) is one of the most popular fish species. According to data from the Ministry of Maritime Affairs and Fisheries (KKP), the export value of goldfish increased by 11% from 2019 to 2020, rising from USD 49,961 in 2019 to USD 55,581 in 2020 (KKP, 2020) [6]. Noviyanti *et al*. (2015) [16] note that goldfish are highly sought after due to their attractive body shape and colors, with the Oranda variety being particularly popular. Saragi *et al*. (2021) [24] describe Oranda goldfish as having berry-like growths (resembling raspberries) that cover almost the entire head, except for the eyes and mouth. A significant issue in goldfish egg production is the low hatching rate, often attributed to improper hatching temperatures and the adhesive nature of the eggs. Previous studies have indicated that low hatching rates are due to unsuitable temperature and environmental conditions (Sandi, 2021; Muslim & Adjo, 2021; Maulana *et al*., 2019) [22, 12, 11]. The adhesive nature of the eggs also hinders their development, necessitating the immersion of goldfish eggs in a tannin solution to reduce adhesiveness. Yustiati *et al*. (2021) [32] explain that the adhesive property of the eggs can obstruct oxygen penetration, thereby disrupting egg development and affecting the hatching rate. Immersing the eggs in a tannin solution is expected to enhance goldfish cultivation. Tannins are compounds that can reduce egg adhesiveness by eroding the mucus layer containing proteins on the egg surface (Prayitno & Nugroho, 2020; Yohanes *et al*., 2022) [19, 30]. According to Widyawati *et al*. (2023) [29], tannins can bind proteins, forming complexes resistant to protease, thus degrading the proteins. In addition to bioactive additives like tannins, temperature is another critical factor affecting egg hatching success. Sahada *et al*. (2022) [21] state that temperature influences the duration of egg hatching, and proper temperature treatment can expedite the process. Sandi & Nursyahran (2021) [22] explain that the most effective temperature for egg hatching is 30 °C. Muslim & Ato (2021) [12] indicate that a temperature range of 22-32 °C is optimal for egg hatching. Sarifudin & Nasmia (2023) [25] note that higher water temperatures can stimulate faster egg hatching, whereas lower temperatures can delay it.

Material and Methods

The equipment and materials used in this study included one male goldfish (*C. auratus*), approximately 1 year old, weighing 110.8 g, and two females, also around 1 year old, weighing 129.4 g and 114.4 g, all of which were healthy with bright coloration. These fish were obtained from Base Camp Mina Papillon. The eggs were collected through artificial spawning, with 100 eggs used per treatment. GnRHA hormone and domperidone, under the brand "Ovaprim," were used for final maturation and egg release in the goldfish, while tannin was used to reduce the adhesiveness of the goldfish eggs. The tannin used was 100% pure powder produced by CV. Sentra Teknosains Indonesia.

The research method used was an experimental approach. The experimental design employed in this study was a completely randomized design (CRD) with 4 treatments and 3 replications. Each replication consisted of 100 eggs, totaling 1,200 eggs, which were soaked in a tannin solution at 6g/L for 4 minutes before being placed into aquariums set to the treatment-specific temperatures. The eggs were then rinsed with clean water and transferred to hatching containers and larval rearing containers for 7 days.

The temperature treatments in this study are as follows:

- **Treatment A:** Temperature 24 °C
- **Treatment B:** Temperature 26 °C
- **Treatment C:** Temperature 28 °C
- **Treatment D:** Temperature 30 °C

Container preparation

Before preparing the broodstock, the rectangular aquarium, measuring 40 cm in length, 30 cm in width, and 30 cm in height, was cleaned. The aquarium was scrubbed thoroughly with a sponge to remove any adhered dirt and then rinsed with running clean water. After rinsing the aquarium until no more dirt remained, it was filled with clean water up to a height of 25 cm, using water from a well. Once the water was added, aeration was provided, and a water heater was installed to regulate the water temperature, along with a thermometer to continuously monitor the temperature.

Preparation of broodstock

The test fish used in this study were 1-year-old goldfish (*C. auratus*) broodstock, consisting of one male weighing 110.8g and two females weighing 129.4 g and 114.4 g, respectively. Female broodstock with mature gonads were identified by their enlarged abdomen, which felt soft and swollen at the bottom upon palpation, and their reddish urogenital area. Male broodstock with mature gonads were identified by performing stripping on the abdomen towards the urogenital opening, which released milky white sperm. The spawning process was carried out by stripping the abdomen of both the female and male broodstock. Stripping was performed after hormone injection to facilitate the release of eggs and sperm. The dosage of sGnRH and dopamine hormone injection for the female broodstock was 0.5 ml/kg, while for the male broodstock it was 0.3 ml/kg. Stripping was conducted 8 hours after injection by gently massaging the abdomen towards the genital area.

Fertilization was done by preparing the eggs and sperm obtained from the stripped broodstock. The sperm was diluted using a physiological NaCl solution to reduce its viscosity and increase its volume. The stripped eggs and diluted sperm were then mixed and stirred with a pigeon feather for 3 minutes to ensure sufficient contact between the eggs and sperm. The number of eggs used in the study was 100 per replicate, with a total of 1,200 eggs needed for the entire experiment.

Immersion and Egg incubation

The fertilized eggs were subjected to a pre-treatment immersion process using a 6g/L tannin solution. The tannin solution was prepared before the fertilization process by dissolving 6g of tannin powder in 1L of distilled water and stirring until the tannin powder was completely dissolved. After fertilization, the eggs were soaked in the 6g/L tannin solution for 4 minutes. During this time, the eggs were gently stirred with a pigeon feather for 4 minutes. After the immertion period, the eggs were carefully rinsed with clean water and placed in hatching containers, which were aquariums filled with water at the specified treatment temperatures and equipped with aeration to supply oxygen. Clear eggs indicated fertilized eggs, while cloudy white eggs indicated unfertilized eggs, which would not hatch. The eggs were then placed in incubation containers, which were aquariums set to treatment temperatures of A (24 °C), B (26 °C), C (28 °C), and D (30 °C), to observe fertilization rate, hatching time, hatching rate, and survival rate.

Larvae maintenance

After the eggs hatched, the larvae were maintained for 7 days or 1 week. Oranda goldfish larvae, 3 days old after their yolk sac reserves were depleted, were fed boiled egg yolk dissolved in mineral water. The feed was administered using a dropper pipette in sufficient amounts. The feeding frequency for the egg yolk was every 3 hours for a total of 15 hours per day.

Fertilization rate

The method used to determine the fertilization rate (FR) of the observed fish gonads involved the following formula. According to Nainggolan *et al.* (2023) [14], fertilized eggs appear shiny yellow, while unfertilized eggs appear cloudy white. The fertilization rate was calculated based on the formula proposed by Rustidja (1997) [20] as follows:

$$FR\ (\%) = \frac{Qt}{Q0} \times 100\%$$

Description

FR: Fertilization rate (%)
Qt: Number of fertilized eggs (units)
Q0: Total number of ovulated eggs (units)

Hatching time duration

The measurement of hatching time duration was conducted by observing the period from when the eggs were fertilized until the beginning of hatching. Observations were made using a sample of 100 eggs for each treatment and replicate until hatching occurred. The observations were conducted in each aquarium by directly checking at intervals of 2-3 hours. Consequently, the data obtained represented an estimated time rather than the real-time hatching time of the eggs.

Hatching rate

According to Ulyana *et al.* (2018) [28], the formula for calculating the hatching rate can be as follows:

HR (%): $\frac{\text{Hatching eggs}}{\text{Total observed eggs}} \times 100$

Description
HR: Hatching rate (%)

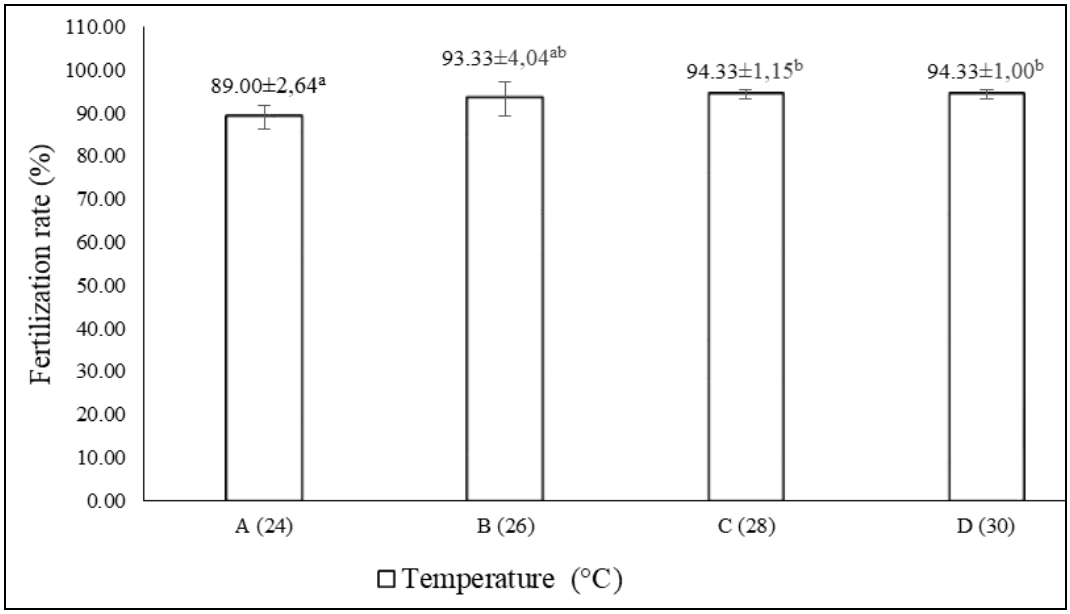
Survival rate
According to Mustofa *et al*. (2018), the survival rate can be measured using the following formula:

SR: $\frac{Nt}{N0} \times 100\%$

Description
SR: Survival rate (%)
Nt: Number of individuals at the end of the study (units)
N0: Number of individuals at the beginning of the study (units)

Water quality
The water quality parameters observed in this study included temperature, dissolved oxygen (DO), and pH. Measurements of temperature, pH, and dissolved oxygen (DO) were conducted three times a day: in the morning, at noon, and in the evening. The temperature was monitored every 2 hours using a thermometer, while a pH meter was used to measure the pH of the water, and a DO meter was used to measure the dissolved oxygen levels in the water.

Result and Discussion
Egg Fertilization Rate
Based on observations conducted during the study, the fertilization rate, or the number of fertilized goldfish eggs (*C. auratus*), was determined, allowing for the calculation of the fertilization rate. The data on the fertilization rate of goldfish eggs are presented in Figure 1.

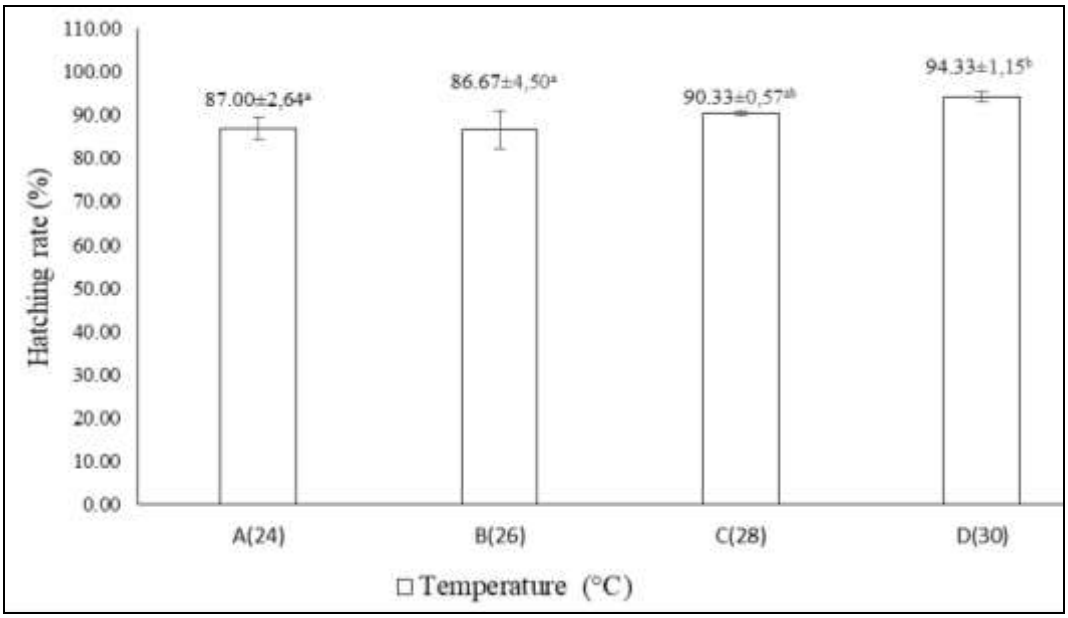


Hatching Duration
Based on observations of the hatching duration of goldfish eggs (*C. auratus*) under different temperature treatments, the hatching duration data can be seen in Table 1.

Table 1: Hatching duration of eggs

	Treatment			
	A (24 °C)	**B (26 °C)**	**C (28 °C)**	**D (30 °C)**
Average (Minute)	3.360	3.300	3.120	3.120

Hatching Rate
The hatching of goldfish eggs (*C. auratus*) was observed for 1-3 days after fertilization. The hatchability of goldfish eggs, obtained from the study using the same tannin dose for egg immersion, is presented in Figure 2.



Survival Rate

The survival rate of goldfish larvae (*C. auratus*) was monitored for 7 days post-hatching. This was assessed by counting the number of larvae remaining during the 7-day rearing period. The survival rate data for goldfish are presented in Figure 3.

Water Quality

Water quality was monitored throughout the study, covering the aquarium conditions from egg hatching to larval rearing of goldfish (*C. auratus*). The observed water quality parameters included temperature, dissolved oxygen (DO), and pH. The measurements of water quality over the study period are presented in Table 2.

Table 2: Water quality

Treatment	Water Quality		
	Temperature (°C)	DO (mg/L)	pH
A	24	5,6-7,5	6,8-8,3
B	26	5,6-7,6	6,8-8,4
C	28	5,6-7,8	6,8-8,5
D	30	5,5-7,6	6,9-8,2
References	25-30 Sutisna & Sutarmono (1995)	>3mg/L Antono (2010); Saragih *et al.* (2018) [24]	6,5-8,5 Yufika *et al.* (2019) [31]

Discussion

Based on research results, goldfish eggs exhibit a high fertilization rate at treatment C (28 °C) with 94.33±1.15%, and treatment D (30 °C) with 97.00±1.00%. Compared to the study by Yufika *et al.* (2019) [31], the average fertilization rate of goldfish eggs without temperature treatment and tannin immersion was 86.73%. Treatments C (28 °C) and D (30 °C) show good values, presumably related to the high temperature, which can help increase the fertilization percentage of goldfish eggs. Muslim *et al.* (2021) [12] stated that too cold or too hot temperatures could inhibit the hatching process, and even extreme or fluctuating temperatures can cause hatching failures or embryo death. The fertilization response of the eggs from this study indicates that temperature affects the fertilization rate of the eggs. According to Siegers *et al.* (2021) [26], the lower the temperature of the water medium, the slower the fertilization time, and the eggs may not be fertilized at all. Eggs that stick together also affect the fertilization rate of goldfish eggs, as adhesive eggs hinder sperm from entering the egg cell, resulting in unfertilized eggs. With the use of tannins in this study, the number of adhesive eggs decreased, thus increasing the fertilization percentage of goldfish eggs. According to Lubis *et al.* (2022) [9], if the eggs stick to each other, the potential for fertilization decreases.

Conversely, if the eggs do not stick to each other, the percentage of fertilized eggs by sperm increases.

Based on observations, the fastest hatching time for goldfish eggs was found in treatment C (28 °C) and treatment D (30 °C), both at 3,120 minutes. The average hatching time for eggs in the study by Ayulandari *et al.* (2023) [3], without temperature treatment and tannin immersion, was approximately 3,240 minutes. The hatching time can be influenced by temperature, as it affects egg development. Higher water temperatures lead to faster hatching, while lower temperatures result in slower hatching. According to Aldillah and Husniati (2023) [1], eggs take longer to hatch at lower temperatures and hatch more quickly at higher temperatures. Ayulandari *et al.* (2023) [3] also explain that hatching is influenced by the presence of substances that enhance the activity of hatching enzymes. The hatching time is also affected by the metabolic rate of the goldfish embryos. Adriyanto *et al.* (2013) and Alfath *et al.* (2020) state that temperature changes significantly impact embryo development by affecting the metabolic rate of the embryos. Temperature reduces the metabolic activity of cells, thereby inhibiting egg development. Consequently, the metabolic rate of the embryos ultimately influences the hatching time of goldfish (*C. auratus*) eggs.

Based on the analysis of variance data for the hatching rate of goldfish eggs, good results were obtained for treatment C (28 °C) at 90.33±0.57% and treatment D (30 °C) at 94.33±1.15%.

It is suspected that the quantity of hatched goldfish eggs depends on the water temperature. Compared to the two highest-value treatments in the study by Manulang (2019), the 30 °C treatment showed a value of 93.67% and the 28 °C treatment showed a value of 77.67%. The high hatching rate values obtained in this study are likely due to the water temperature being within the normal range for goldfish and the use of tannin immersion treatment on the goldfish eggs. According to Muslim *et al*. (2021) [12], the optimal temperature range for goldfish is between 22 °C and 32 °C. Higher temperatures can increase the metabolic process of the eggs, giving them a greater chance to hatch. Pakpahan *et al*. (2024) [17] state that temperature plays an important role in gametogenesis, supporting the success of egg hatching. Factors that can affect the hatching rate include eggs sticking together, which prevents optimal oxygen circulation, making the role of tannin in reducing the percentage of adhesive eggs crucial in increasing the hatching rate. According to Lubis E. S. *et al*. (2022) [9], adhesive eggs cannot receive oxygen properly, leading to egg damage and fungal growth. This is also supported by Khosim *et al*. (2023) [7], who state that factors causing eggs not to hatch include eggs sticking together, which hampers optimal oxygen circulation. Fitriana *et al*. (2021) [5] also state that when fish eggs stick together during spreading, it disrupts oxygen circulation, leading to oxygen deficiency and death of the eggs.

The percentage of the survival rate (SR) of goldfish during the 7-day research period showed good results in treatment C (28 °C) at 83.67±0.57% and treatment D (30 °C) at 88.33±4.16%. The high survival rate in this study is likely due to the significant effect of temperature on the survival rate of goldfish. Niron *et al*. (2023) [15] stated that a normal temperature range plays a role in growth and survival. Based on the research by Laila & Purwasih (2020) [8], the average survival rate of Oranda goldfish larvae ranged from 30.49% to 48.46%, as their study did not use temperature treatment or tannin immersion. The survival rate obtained from research that did not use temperature treatment or pre-treatment by immersing goldfish eggs in a tannin solution was 76.9% (Dinda & Aminullah, 2024) [4]. Factors that can affect survival include eggs sticking together, so the pre-treatment of immersing eggs in a tannin solution used in this study can also result in a high survival rate. According to Patricius *et al*. (2019) [18], the removal of the mucus layer (adhesiveness) on the egg surface, and eggs not sticking to each other, which prevents covering the micropyle (the entrance for oxygen), can increase the survival rate. With eggs not sticking to each other, the hatched larvae will not struggle or get trapped in adhesive eggs.

During the study, water temperatures ranged from 24 °C to 30 °C, dissolved oxygen (DO) levels ranged from 5.5 to 7.5 mg/L, and pH ranged from 6.9 to 8.3. These conditions were stable and optimal for goldfish life and growth. According to SNI (2017), the optimal temperature range is between 26 °C and 30 °C, with DO above 5 mg/L and pH between 6.5 and 8. Sutisna and Sutarmono (1995) indicated that the temperature required for hatching is between 25 °C and 30 °C. Besides temperature, the DO levels throughout the study were also optimal. According to Antono (2010); Saragih *et al*. (2018) [24], the optimal range for dissolved oxygen in goldfish rearing is >3 mg/L. The pH range observed in the study was also within the optimal range. Yufika *et al*. (2019) [31] noted that the ideal pH for goldfish is between 6.5 and 8.5.

Conclusion

Based on the research conducted, the following conclusions can be drawn

1. Tannin immersion prior to incubation and incubation temperature significantly affect the hatching rate and survival rate of goldfish (*C. auratus*) eggs.
2. Treatments C and D yielded favorable results, with Treatment C achieving a hatching rate of 90.33±0.57% and a survival rate of 83.67±0.57%, while Treatment D achieved a hatching rate of 94.33±1.15% and a survival rate of 88.33±4.16%. Treatment C and D produced the same good results, but it is recommended that farmers use Treatment C because it requires less cost.

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