

# The effect of dose and immersion time of *Camellia sinensis* solution on the egg adhesion and hatching of *Pangasianodon hypophthalmus*

## Pengaruh dosis dan lama perendaman larutan *Camellia sinensis* terhadap daya rekat dan penetasan telur *Pangasianodon hypophthalmus*

Aldi Baihaki, Nur Asiah\*, Nuraini Nuraini

Department of Aquaculture, Faculty of Fisheries and Marine Science, Riau University, Pekanbaru, Riau 28293, Indonesia

\*Corresponding author: nur.asiah@lecturer.unri.ac.id

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### ABSTRACT

*Pangasianodon hypophthalmus*'s cultivation is growing rapidly, but there was a problem with the egg's adhesiveness. Overcome this problem given a tea solution that serves as a remove of *Pangasianodon hypophthalmus* egg adhesion. The purpose of this study was evaluating the effect of *Camellia sinensis* immersion with different doses and immersion duration on *Pangasianodon hypophthalmus* egg's adhesion. This study used a Completely Randomized Design Factorial with two factors. The first factor was the dose (g/L) of the black tea, with 4 levels; 0 (D0), 8 (D8), 10 (D10), and 12 (D12). The second factor was the immersion duration (minutes) with three levels of 3 (W3), 4 (W4), and 5 (W5). *Pangasianodon hypophthalmus* larvae reared for seven days. Parameters measured included egg adhesion, fertilization rate, hatching rate, survival rate, and water quality. The result showed that the *C. sinensis* solution's doses had a significant effect ( $P < 0.05$ ) on the fertilization and hatching rate of *Pangasianodon hypophthalmus* eggs. The optimum treatment with a dose of 10 g/L and four minutes (D10W4) resulted in increased eggs adhesion 5.99%, fertilization rate 94.13%, hatching rate 92.88%, and survival rate 96.99%. The water quality during the study was temperature 26.7-27.9°C, pH of larvae maintenance water 6.5-7.1 and DO 4.0-5.2 mg/L. There was an interaction between dose and immersion duration of *C. sinensis* solution to the egg adhesion and hatching eggs of *Pangasianodon hypophthalmus*.

Keywords: black tea, hatching rate, survival rate, striped catfish

### ABSTRAK

Budidaya ikan patin siam berkembang pesat, namun masalah yang dihadapi adalah telur ikan yang merekat. Mengatasi masalah ini diberikan larutan teh yang berfungsi sebagai penghilang daya rekat telur ikan patin siam. Tujuan dari penelitian ini adalah untuk mengetahui pengaruh perendaman telur dengan dosis dan lama perendaman yang berbeda pada larutan teh hitam (*Camellia sinensis*) terhadap daya rekat dan penetasan telur ikan patin siam. Penelitian ini menggunakan Rancangan Acak Lengkap (RAL) Faktorial dengan dua faktor. Faktor pertama adalah dosis larutan teh hitam (g/L) dengan 4 taraf; 0 (D0), 8 (D8), 10 (D10), dan 12 (D12). Faktor kedua adalah lama perendaman (menit) dengan 3 taraf yaitu 3 (W3), 4 (W4), dan 5 (W5). Larva ikan patin siam dipelihara selama tujuh hari. Parameter yang diukur adalah daya rekat telur, nilai pembuahan, nilai penetasan, kelulushidupan, dan kualitas air. Hasil penelitian menunjukkan bahwa dosis dan lama perendaman larutan teh hitam (*C. sinensis*) berpengaruh nyata ( $P < 0,05$ ) terhadap daya rekat, nilai pembuahan dan nilai penetasan telur ikan patin siam. Perlakuan yang terbaik dengan dosis 10 g/L dan waktu perendaman empat menit (D10W4) memberikan daya rekat telur 5,99%, nilai pembuahan 94,13%, nilai penetasan 92,88%, dan kelulushidupan 96,99%. Kualitas air selama penelitian adalah suhu 26,7-27,9°C, pH air pemeliharaan larva 6,5-7,1 dan DO 4,0-5,2 mg/L. Terdapat interaksi antara dosis dan lama perendaman larutan teh hitam (*C. sinensis*) terhadap daya rekat telur dan penetasan telur ikan patin siam

Kata kunci: ikan patin siam, kelulushidupan, nilai penetasan, teh hitam

## INTRODUCTION

Stripped catfish (*Pangasionodon hypophthalmus*) is one of the freshwater fish commodities which is the center for the development of fisheries commodities in Riau Province. Stripped catfish have the advantage that can be reared with high stocking densities, minimal use of water, easy to cultivate, have economic value, and are much consumed by the community (Septimesy *et al.*, 2016). The high potential of this fish causes an increase in demand so cultivation activities require continuous availability of the quantity and quality of seeds. One way to meet the availability of these seeds is to carry out catfish hatchery activities.

However, in the hatchery of Stripped catfish there are often problems that cause low hatchability of catfish eggs. The problem in hatching catfish eggs is that Stripped catfish eggs are adhesive. The adhesive nature of eggs causes egg hatchability to be low. The protein layer that causes the eggs to stick together is formed around the vitelline layer which is composed of glucoprotein (Yustiati *et al.*, 2021). Several studies have been conducted to remove the stickiness of eggs using natural ingredients, as was done by Fani *et al.* (2018) using clay, termite soil (Dewi & Widita, 2015), pineapple solution (Patricius *et al.*, 2019), papaya latex (Saputra *et al.*, 2014) and Situmorang *et al.* (2021) used a powdered milk solution which was able to remove the adhesion of catfish eggs by 76.98% while without giving powdered milk it was 32.65%. Giving a solution that functions as a remover of egg adhesion aims to increase higher egg hatching. One of the potential natural ingredients used is black tea solution.

Black tea is a type of tea (*Camellia sinensis*) which is fermented using polyphenol oxidase enzymes. The potential content of black tea as an alternative ingredient is to remove the stickiness of fish eggs because tea contains tannin compounds. The most tannin content contained in tea is 30% (Yustiati *et al.*, 2021). The tannin content in the black tea solution is able to degrade the glucoprotein coating on Stripped catfish eggs. The glucoprotein coating on Stripped catfish eggs can be degraded by tannin compounds by binding and precipitating a number of protein molecules that bind to each other and become complex compounds, namely tannins-proteins so that they can reduce the adhesion of the eggs (Badarullah *et al.*, 2020).

The research that use of black tea solution has been carried out by Hasyim (2016) using the best dose of black tea solution at a dose of 10 g/L on African catfish resulting an egg adhesion of 23.33%. Research conducted by Yustiati *et al.* (2021) using the best dose of black tea solution was carried out at a dose of 10 g/L with a immersion time of four minutes for sangkuriang catfish resulting in an egg adhesion of 12%. Research by Rahayu (2015) showed the adhesion of African catfish eggs (*Clarias gariepinus*) obtained in four minutes immersion of 64% and 81.21% hatchability. Based on the above, it is necessary to conduct research on the effect of the dose and immersion time of black tea solution on the adhesion and hatching of Stripped catfish eggs.

## MATERIALS AND METHODS

### Time and place

This research was conducted in June 2022 at the Fish Hatchery and Breeding Laboratory, Faculty of Fisheries and Marine, University of Riau, Pekanbaru.

### Breeder test

Stripped catfish parent (*Pangasionodon hypophthalmus*) broodstock were obtained from the local hatchery unit in Pekanbaru, Riau as much as 1 pair with the gonadal maturity level at TKG IV with a female parent weight of 3.30 kg and 0.45 kg male parent. The test eggs used were eggs and larvae resulting from artificial spawning with ovaspec stimulation on Stripped catfish conducted at the Fish Hatchery and Breeding Laboratory (PPI), Faculty of Fisheries and Marine, University of Riau. Eggs were spread in an aquarium measuring 30×30×30 cm<sup>3</sup> as much as 1 g (596 points).

### Stimulants

The hormone used in this study was sGnRHa+Domperidone with the trademark ovaspec. The Ovaspec used is packaged in a small 10 ml bottle produced by Spectrum Asia PLT Malaysia. Inoculation of the female catfish was done twice by injecting on the back of the fish, in the first injection it was injected 1/3 part and in the second injection 2/3 part with the time interval between the first and second injections for six hours. Injection the second at the same time as the injection male parent (Fani *et al.*, 2018).

Before injection, the parent is weighed first to determine the dose of ovaspec used. Parent used as much as 1 pair.

### Container sterilization solution

Potassium permanganate (PK) with the chemical formula  $\text{KMnO}_4$  as a powder as well as a violet colored solution which is useful for preventing the growth of fungi or bacteria, especially in the fish hatchery process (Karwani & Setyogati, 2019). The solution used was a solution of PK (Potassium Permanganate) which was dissolved in 1 mg/L for 24 hours. Potassium permanganate is a chemical compound that can be used as a disinfectant to prevent bacterial and viral infections (Kuswiyanto, 2015).

### Black tea solution

The tea used in this study was commercially available black tea from Kayu Aro, Solok Regency, West Sumatra. First, boil the water until it boils to 4 liters, then the boiling water is dipped in the tea according to the dose for eight minutes (Hasyim, 2016)

### Research methods

The method used was an experimental method with a completely randomized design (CRD) of two factors, the first factor with different doses of black tea consisting of 4 levels; namely control, 8, 10 and 12 g/L. The second factor was the difference in immersion time which consisted of 3 levels of 3, 4 and 5 minutes respectively. To minimize errors, each treatment level was repeated three times. Determination of dose refers to Hasyim (2016), while the long immersion treatment refers to Rahayu (2015).

D0W0: Control (Immersion without black tea solution)

D8W3: Treatment dose of 8 g/L and immersion time of 3 minutes

D8W4: Treatment dose of 8 g/L and immersion time of 4 minutes

D8W5: Treatment dose of 8 g/L and immersion time of five minutes

D10W3: Treatment dose of 10 g/L and immersion time of 3 minutes

D10W4: Treatment dose of 10 g/L and immersion time of 4 minutes

D10W5: Treatment dose of 10 g/L and immersion time of 5 minutes

D12W3: Treatment dose of 10 g/L and immersion time of 3 minutes

D12W4: Treatment dose of 10 g/L and immersion time of 4 minutes

D12W5: Treatment dose of 10 g/L and immersion time of 5 minutes

### Research procedure

#### Container preparation

The preparation of the container starts from the cleaning process of the egg immersion container, namely a basin with a diameter of 30 cm, the hatching container, which is an aquarium measuring  $30 \times 30 \times 30 \text{ cm}^3$ . The immersion and hatching containers were first sterilized using PK solution at a dose of 1 mg/L. Then rinse and dry. After the container is dry, fill 2 L of water in the basin and fill with water as high as  $\pm 11 \text{ cm}$  or 10 L of water in the hatching container.

#### Spawning of stripped catfish

Spawning was carried out artificially with hormone doses of 0.2 mL/kg male parent and 0.5 mL/kg female parent. Before the injection the broodstock was weighed first to determine the dose of ovaspec used, 1 pair of broodstock was used.

#### Making black tea solution

Black tea weighing 2 g/pack, first boil the water until it boils. Then take 2 L of boiling water, dip the tea according to the dose and stir until evenly distributed. Making a tea solution at a dose of 8 g/L using 16 g (8 packs) of tea, at a dose of 10 g/L using 20 g (10 packs) of tea and at a dose of 12 g/L using 24 g (12 packs) with a volume of 2 liters of water per treatment. The tea solution was prepared at  $100^\circ\text{C}$  so that the essence of the tea can dissolve completely in the water. The preparation of the black tea solution was carried out 1 hour before the process of stripping the Stripped catfish mains.

#### Fertilization

The eggs and sperm that have been obtained are mixed in a bowl and a physiological solution is added to activate the fish sperm. Eggs and sperm are stirred with chicken feathers and then rinsed with a fertilization solution made from a mixture of 3 g of urea and 4 g of NaCl dissolved in 1 L of distilled water. Next, the eggs are placed in the immersion container using chicken feathers to be immersed in the tea solution with the dose and duration of immersion according to the treatment used.

### Immersion eggs with black tea solution

First prepared a container in the form of a basin with a diameter of 30 cm as many as 30 units and labeled it according to the treatment. The eggs that have been fertilized are then taken as much as 1 g (596 eggs). After that, put the filter where the eggs were spread into black tea solution basin. Then spread 1 g of eggs that have been fertilized in the filter. Then for the process of immersion eggs is carried out according to the predetermined immersion time. After immersion, then the eggs are soaked in clean water so that there are no eggs black tea solution the remaining. Then transferred to the hatchery, observed the adhesion of the eggs to determine the reduction in the adhesive properties of the eggs.

### Embryogenesis stripped catfish eggs

Catfish eggs were taken using chicken feathers to observe embryogenesis. The treatments observed for embryogenesis were the control treatment and the treatment at a dose of 10 g/L. The collected eggs were placed in a petri dish and observed under an Olympus microscope using 10×4 magnification. The time of each embryo phase was recorded and documented during the observation. Observations were made until the fish eggs hatched.

### Hatchability

Nuraini *et al.* (2013) stated that for the counting of fertilized eggs, after 9-10 h of fertilization, then the unfertilized eggs will turn milky white and are discarded, then the eggs are incubated again and the egg hatching count is carried out 9-10 h after the eggs begin to hatch.

### Larvae Care

After the eggs hatch, the larvae are reared for seven days. The feed given is artemia at the age of 3-5 days and silkworms (*Tubifex* sp.) was given ad libitum with the frequency of feeding four times a day. After seven days of maintenance, the percentage of larval survival during rearing was calculated. Water quality measurements (temperature, pH, DO) were carried out at the beginning, middle and end of the study.

### Measured Parameters

#### Egg Adhesive

$$\text{Egg adhesion} = \frac{\text{Number of glued egg}}{\text{Initial number of glued egg}}$$

#### Fertilization rate (FR)

The calculation of percentage of eggs that were fertilized in each treatment was calculated using the formula proposed by Esa *et al.* (2023) as follows:

$$\text{FR (\%)} = \frac{\text{Number of fertilized eggs}}{\text{Number of estimated eggs}} \times 100$$

#### Hatching rate (HR)

The calculation of percentage of eggs that were fertilized in each treatment was calculated using the formula proposed by Esa *et al.* (2023) as follows:

$$\text{HR (\%)} = \frac{\text{Total number of hatched eggs}}{\text{Total number of fertilized eggs}} \times 100$$

#### Survival rate (SR)

Larval survival during rearing can be calculated using the formula:

$$\text{SR (\%)} = \frac{\text{Total number of larvae} - \text{Number of dead larvae}}{\text{Total number of larvae}} \times 100$$

## RESULTS AND DISCUSSION

### Result

*Effect of different doses and immersion times on adhesion, fertilization value, hatching value and survival of stripped catfish larvae (Pangasionodon hypophthalmus)*

The effect of the dose of black tea solution (*Camellia sinensis*) and different immersion times on adhesion (%), fertilization value (%), hatching value (%) and survival rate (%) are presented in Table 1.

*Effect of different doses and immersion time on the adhesiveness of stripped catfish eggs (Pangasionodon hypophthalmus)*

The results of the adhesion of Stripped catfish eggs at different doses and immersion times using black tea solution are presented in Figure 1.

*The effect of different doses and immersion time on the fertilization rate of stripped catfish eggs (Pangasionodon hypophthalmus)*

The effect of the dose of black tea solution (*Camellia sinensis*) and different immersion times with respect to fertilization values are presented in Figure 2.

Table 1. Effect of different doses of black tea solution and immersion time on adhesion (%), fertilization value (%), hatching value (%), and survival (%) of Stripped catfish larvae (*Pangasionodon hypophthalmus*).

Dose and Time	Egg Adhesion (%)	Fertilization Rate (%)	Hatch Rate (%)	Survival Rate (%)
D0W0	74.83 ± 1.27 <sup>i</sup>	75.45 ± 1.26 <sup>a</sup>	64.26 ± 1.00 <sup>a</sup>	96.65 ± 0.29 <sup>a</sup>
D8W3	39.15 ± 1.50 <sup>h</sup>	87.53 ± 1.12 <sup>c</sup>	78.47 ± 0.96 <sup>cd</sup>	96.66 ± 0.15 <sup>a</sup>
D8W4	35.74 ± 0.34 <sup>g</sup>	88.31 ± 0.76 <sup>c</sup>	80.25 ± 1.19 <sup>d</sup>	96.76 ± 0.12 <sup>a</sup>
D8W5	28.30 ± 1.61 <sup>f</sup>	90.38 ± 0.95 <sup>d</sup>	83.91 ± 1.33 <sup>e</sup>	96.68 ± 0.21 <sup>a</sup>
D10W3	18.01 ± 0.26 <sup>e</sup>	91.39 ± 0.98 <sup>of</sup>	88.44 ± 1.36 <sup>f</sup>	96.96 ± 0.12 <sup>a</sup>
D10W4	15.10 ± 1.26 <sup>d</sup>	94.13 ± 1.21 <sup>f</sup>	92.88 ± 1.34 <sup>g</sup>	96.99 ± 0.22 <sup>a</sup>
D10W5	11.58 ± 1.27 <sup>c</sup>	93.35 ± 0.76 <sup>ef</sup>	90.42 ± 0.97 <sup>fg</sup>	96.95 ± 0.10 <sup>a</sup>
D12W3	9.67 ± 1.27 <sup>bc</sup>	86.30 ± 0.70 <sup>c</sup>	75.71 ± 2.06 <sup>bc</sup>	96.65 ± 0.32 <sup>a</sup>
D12W4	7.94 ± 1.63 <sup>ab</sup>	83.39 ± 1.21 <sup>b</sup>	73.66 ± 2.09 <sup>b</sup>	96.72 ± 0.05 <sup>a</sup>
D12W5	5.99 ± 1.02 <sup>a</sup>	82.94 ± 2.02 <sup>b</sup>	72.45 ± 3.53 <sup>b</sup>	96.75 ± 0.32 <sup>a</sup>

Note: Uppercase letters behind the mean ( $\pm$ standard deviation) in the same row indicate a significant difference ( $P < 0.05$ ).

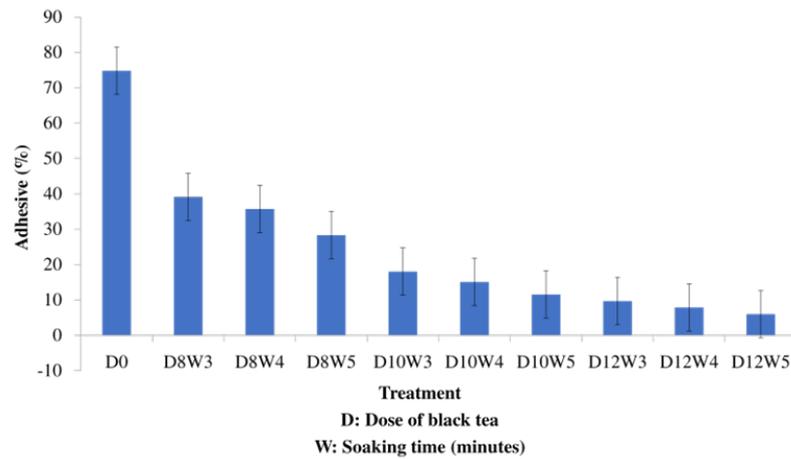
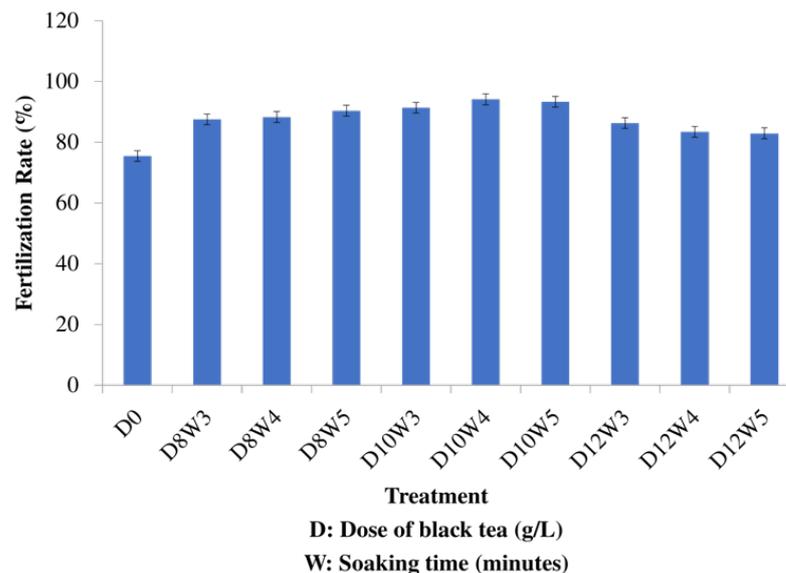
Figure 1. Histogram effect of different doses and immersion times on the adhesion of Stripped catfish eggs (*Pangasianodon hypophthalmus*).

Figure 2. Histogram the effect of different doses and immersion times on the fertilization value of Stripped.

### Embryo Development

Based on observations of the developmental time of Stripped catfish embryos (*Pangasianodon hypophthalmus*) that have been carried out are presented in Table 2.

### Effect of different dose and immersion time on hatching rate of stripped catfish eggs (*Pangasionodon hypophthalmus*)

The effect of the dose of black tea solution (*Camellia sinensis*) and immersion time differ with respect to hatching values presented in Figure 3.

Table 2. Fish Embryo Development Time *P. Hypophthalmus*.

Embryogenesis Phase	Observation time			
	D0W0 (0 g/L, 0 Minutes)		D10W4 (10 g/L, 4 Minutes)	
	Jam	Minute	Jam	Minute
Conception	0	0	0	0
Blastodic	0	6	0	4
Division I (2 cells)	0	20	0	16
Division II (4 cells)	0	34	0	30
Division III (8 cells)	0	53	0	50
IV division (16 cells)	1	12	1	08
V division (32 cells)	1	38	1	35
Morula	2	06	2	04
Blastula	4	22	4	18
gastrula	5	35	5	32
Perisai Embryo	6	48	6	44
Organogenesis	11	50	11	45
Hatch	21	36	21	30

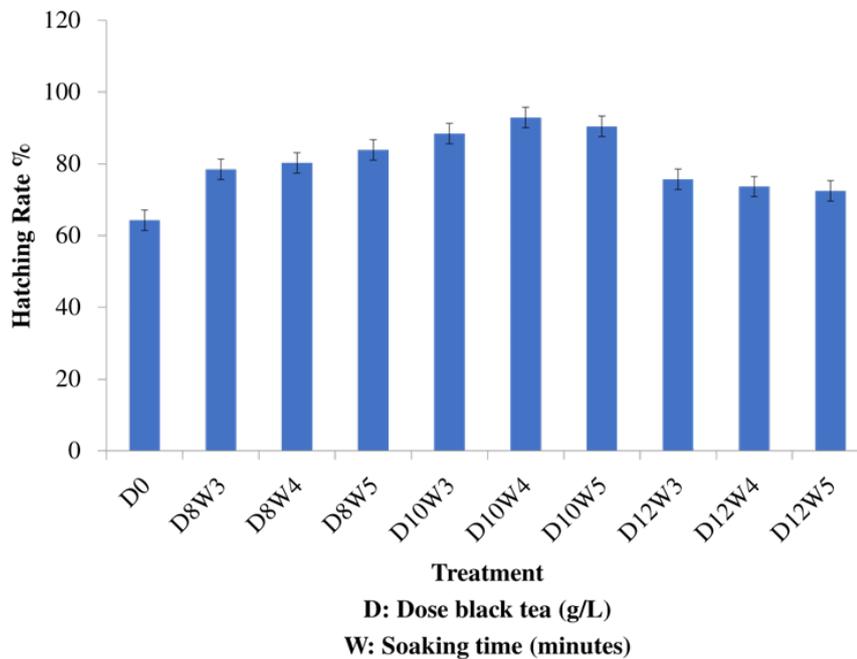


Figure 3. Histogram the effect of different doses and immersion times on the hatching value of Stripped catfish eggs (*Pangasionodon hypophthalmus*).

*Effect of different doses and immersion time on the survival of stripped catfish larvae (*Pangasionodon hypophthalmus*)*

The effect of the dose of black tea solution (*Camellia sinensis*) and different immersion times with respect to survival are presented in Figure 4.

#### Water quality

Water quality parameters measured during the study were temperature, pH and dissolved oxygen (DO). The results of measuring water quality parameters during the study are presented in Table 3.

#### Discussion

##### Effect of different doses and immersion time on the adhesiveness of stripped catfish eggs (*Pangasionodon hypophthalmus*)

Based on Table 1 it is known that the adhesion of Stripped catfish eggs ranges from 5.99% to 74.83%, the results of the Analysis of Variance Test (ANOVA) showed that there was an effect of the dose and immersion time of the black tea solution, and the interaction between the dose and

the immersion time on the adhesion ( $P < 0.05$ ). Based on Figure 1, it is known that the adhesion of Stripped catfish eggs showed the lowest percentage in treatment D12W5 (dose of 12 g/L and five minutes) which was 5.99% while the highest adhesion was found in treatment D0W0 (dose of 0 g/L and time 0 minutes) that is equal to 74.83% of the eggs are still attached.

The D12W5 treatment (dose of 12 g/L and immersion time of five minutes) showed the lowest percentage of eggs that still adhered to each other, namely 5.99%. It is assumed that the higher the concentration in the tea solution, the higher the tannin content so it is more effective in removing the stickiness of the eggs. The tannin content in the black tea solution is able to degrade the glucoprotein coating on Stripped catfish eggs. The glucoprotein coating on Stripped catfish eggs can be degraded by tannin compounds by binding and precipitating a number of protein molecules that bind to each other and become complex compounds, namely tannins- proteins so that they can reduce the adhesion of the eggs (Badarullah *et al.*, 2020).

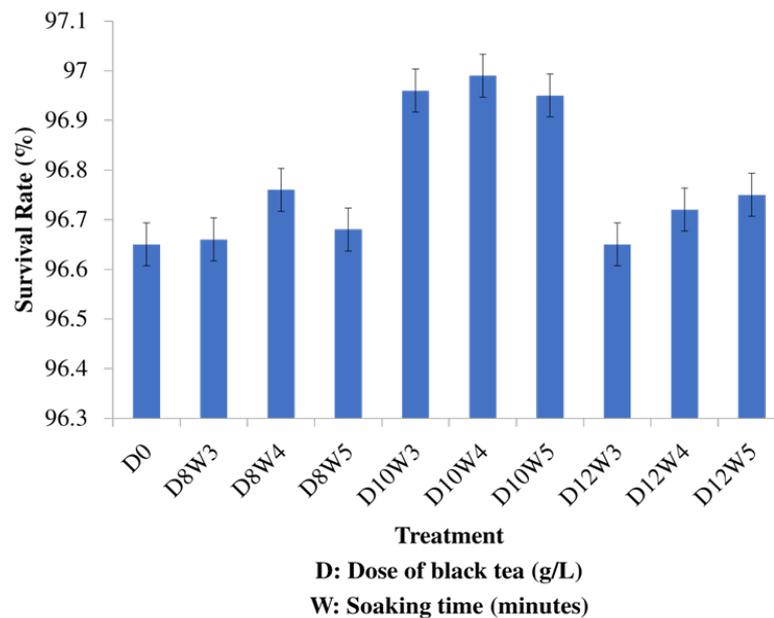


Figure 4. Histogram of the effect of different doses and immersion time on the survival of stripped catfish larvae (*Pangasionodon hypophthalmus*) age seven days.

Table 3. Data on the results of water quality measurements for rearing Stripped catfish larvae.

Parameter	Water quality		
	Early	Middle	End
Temperature (°C)	27.7-27.9	27.4-27.9	26.7-27.4
pH	6.5-7.1	6.7-6.8	6.5-6.6
DO (mg/L)	5.0-5.2	4.5-5.2	4.0-4.2

The results of this study are in line with research by Hasyim (2016) which found that eggs soaked in black tea solution at a dose of 10 g/L with a immersion time of five minutes can reduce the egg adhesive of African catfish eggs by 23.33% and the research of Yustiati *et al.* (2021) showed that using a black tea solution at a dose of 10 g/L for four minutes of immersion time could reduce the stickiness of sangkuriang catfish eggs by a value of 12%. It is presumably, this has occurred due to the breakdown of the adhesive layer by tannin compounds in the tea solution which have the function of binding to proteins and turning them into tannin protein complex compounds. The results of this study indicate that the adhesion of Stripped catfish eggs is 5.99% lower than the results of research by Hasyim (2016) and Yustiati *et al.* (2021). This is presumably with a dose of 12 g/L and immersion time of five minutes, the tannin content in the black tea solution is higher and the immersion time is longer, so that it is more effective in reducing the stickiness of the eggs.

On D0W0 treatment (dose of 0 g/L and time of 0 minutes) resulted in an adhesion value of 74.83% of the eggs sticking together. The high adhesion in this study was because the eggs were not treated with immersion in a black tea solution containing tannin so that the eggs were still adhesive. The adhesive properties of eggs are due to the presence of a glucoprotein layer on the surface of the egg resulting in the eggs potentially sticking to each other or to the substrate through the sticky mucous membrane covering the entire surface of the egg (Slembrouck *et al.*, 2005). The results of this study showed D0W0 (dose 0 g/L and time 0 minutes) in line with several researchers who showed that the highest adhesion was 95% on Stripped catfish eggs (Patricius *et al.*, 2019), in catfish eggs of 95.09% (Situmorang *et al.*, 2021).

### **The Effect of Different Doses and Immersion Time on the Fertilization Rate of Stripped Catfish Eggs (*Pangasionodon hypophthalmus*)**

Based on Table 1 it is known that the fertilization rate of Stripped catfish ranges from 75.45% to 94.13%, the Analysis of Variance Test (ANOVA) showed that there was an effect of the dose and immersion time of the black tea solution, and the interaction between the dose and the immersion time on the fertilization value ( $P < 0.05$ ). The results showed that the percentage of fertilization value in each treatment was caused by the concentration of tannin content and the length of immersion of the eggs. The higher the

concentration of tannin compounds, the more glucoprotein layers are degraded by tannins (Hasyim, 2016). At treatment D10W4 (dose 10 g/L and time four minutes) obtained the highest fertilization value of 94.13%.

This study showed that the interaction between doses of 10 g/L and an immersion time of four minutes was the best treatment for the fertilization value of Stripped catfish eggs. It is suspected that eggs soaked in black tea solution containing tannins can degrade the glucoprotein layer so that the opportunity for spermatozoa to enter the microphyll hole is greater. And it is suspected that tannins are able to bind proteins optimally and tannins are able to hydrolyze the glucoprotein layer so that sperm can fertilize the egg. The results of this study are in line with the sangkuriang catfish eggs soaked in black tea solution at a dose of 10 g/L with a immersion time of four minutes resulting in a fertilization value of 90% (Yustiati *et al.*, 2021), and research Badarullah *et al.* (2020), in asian redbtail catfish eggs of 80.67%. This is suspected by the loss of the adhesive layer which causes the opportunity for cells to divide and develop undisturbed by a lack of oxygen intake.

According to Nainggolan *et al.* (2015), the process of fertilization in the egg is strongly influenced by the quality of the egg, the quality of the sperm and the speed of the sperm to move spontaneously so that they can enter the microfil hole in the egg. The D12W5 treatment (dose of 12 g/L and immersion time of five minutes) produced the lowest fertilization value when compared to other treatments, namely 82.94%. However, treatment D12W5 (dose of 12 g/L and immersion time of five minutes) was higher than the other treatments D0W0 (dose of 0 g/L and time of 0 minutes). It is suspected that the level of fertilization increases with the duration of immersion, but the higher the dose of black tea solution, the more tannins and other active compounds it contains. This causes excessive egg degradation which results in damaged eggs.

At high concentration, tannic caused the chorion to harden, thus decreasing the chorionase activity and the development of embryogenesis is disrupted (Kareem *et al.*, 2017). High concentration of tannic acid gave a negative impact on the pikeperch larvae hatching has been reported by Zakes *et al.* (2005). The D0W0 treatment (dose of 0 g/L and time of 0 minutes) showed the lowest percentage of fertilization value, namely 75.45%. This is presumably because the eggs did not get

the black tea solution treatment which caused the eggs to stick to one another resulting in sperm not being able to penetrate the egg's microphyll hole making it difficult for fertilization to occur (Hasyim, 2016). In general, the success rate of fertilization can be influenced by several factors.

The factors that affect the number of fertilized eggs are water quality, especially DO. In addition, it is also due to adhesive properties that form colonies/clumps, causing death due to lack of oxygen (Effendi *et al.*, 2015). This is because the oxygen supply is needed by the egg at the stages of cell division. This is supported by the opinion of Wild *et al.* (2023) in the oxygen demand can affect the embryonic development and hatching success.

### Embryo Development

Based on the results of observing the fastest embryo development and hatching soaked in black tea solution resulted in the fastest hatching time in the D10W4 treatment (dose 10 g/L, four minutes), namely hatching at an incubation time of 21 hours 30 minutes and the control treatment D0W0 (dose 0 g/L, without immersion), namely hatching at an incubation time of 21 hours 36 minutes. This is presumably due to the presence of a solution that can eliminate the stickiness of the eggs, so that the eggs do not stick together and the eggs get the oxygen they need in the process of embryogenesis properly (Baharudin *et al.*, 2016). Treatment D10W4 (dose of 10 g/L for four minutes) was the best treatment with a faster embryo development and hatching time compared to other treatments, black tea solution at a dose of 10 g/L and immersion time of four minutes could dissolve or hydrolyze mucous membranes which contains glycoproteins in the chorion layer which causes egg adhesion. The results of this study are in line with research by Situmorang *et al.* (2021) Catfish eggs soaked in urea resulted in a hatching time of 21 hours and 10 minutes.

Furthermore, the incubation time in the control treatment without immersion in the black tea solution was longer than in the D10W4 treatment, this was due to the eggs without the immersion in the black tea solution still having a mucous membrane containing glycoprotein in the chorion layer which causes egg adhesion. This is in line with research Situmorang *et al.* (2021) eggs without immersion resulted in a hatching time of 21 hours 35 minutes. The stages of development of embryogenesis into larvae start from the phase cleavage (cell division), morula, blastula,

gastrula, organogenesis until the embryo hatches and comes out of the eggshell.

### Effect of Different Dose and Immersion Time on Hatching Rate of Stripped Catfish Eggs (*Pangasionodon hypophthalmus*)

Based on Table 1 it is known that the hatching value of Stripped catfish ranges from 64.26% to 92.88%, the Analysis of Variance Test (ANOVA) showed that there was an effect of the dose and immersion time of the black tea solution, and the interaction between the dose and the immersion time on the hatching value ( $P < 0.05$ ). The highest hatching value of Stripped catfish eggs was found in treatment D10W4 (dose of 10 g/L and time of four minutes) which was 92.88% while the lowest hatching value was found in D0W0 (dose of 0 g/L and time of 0 minutes) that is equal to 64.26%. Treatment D10W4 (dose of 10 g/L and time of four minutes) showed that the interaction between doses of 10 g/L and immersion time of four minutes was the best treatment for hatching value of 92.88%. This was presumably because the dose in treatment D10W4 (10 g/L) was able to reduce the stickiness of the eggs due to the tannin content and the immersion time of four minutes caused many eggs to be fertilized resulting in the highest hatching rate and was influenced by sufficient oxygen supply for metabolic processes.

At four minutes of immersion, tannins can reduce protein optimally so that the egg's adhesion decreases and the oxygen supply around the egg is sufficient to carry out metabolic processes so that energy is generated which is used to break the eggshell mechanically, starting with the tail coming out of the shell first (Yustiati *et al.*, 2021). This research is in line with research Baharudin *et al.* (2016), immersion asian redbtail catfish eggs in tea solution at a dose of 6 g/L and immersion time of four minutes is 76.67% and research Badarullah *et al.* (2020) immersion with a solution of 6 g/L of tea leaves resulted in the highest hatching value of 78.40%. The results of this study showed that the hatching value of Stripped catfish was 92.88% higher when compared to the results of research by Baharudin *et al.* (2016) and Badarullah *et al.* (2020). This is presumably with a dose of 10 g/L and immersion time off our minutes higher tannin content and optimal immersion time so that it is more effective in increasing egg hatchability.

Treatment D12W5 (dose 12 g/L and time five minutes) decreased hatching value of 72.45%, but higher than treatment D0W0 (dose 0 g/L and time 0 minutes) which was 64.26%. It is suspected

that the high concentration of black tea solution exceeding the maximum limit will reduce the protein contained in the egg coating excessively, causing the chorion layer in the egg to thin which will result in the egg breaking easily and dying. This statement is in accordance with the statement of Taiz & Zeiger (2002), that tannin at the right concentration can be useful in reducing protein, but at excessive levels it can cause damage to the chorion layer. This is caused by shrinkage therefore fluid comes out of the egg and ultimately results in egg death (Putri *et al.*, 2022).

According to Zakes *et al.* (2005), high tannic acid concentrations have an impact on low egg hatching rates. According to statement Tumanung *et al.* (2015), that a high fertilization rate will be followed by a high hatching rate, thus the hatching rate of each treatment follows the fertilization rate. This is in line with research Yustiati *et al.* (2021), immersion of eggs in black tea solution at a dose of 12 g/L and a immersion time of four minutes shows a hatching value of 49%. The D0W0 treatment (dose 0 g/L and time 0 minutes) showed the lowest hatching value compared to other treatments, namely 64.26%.

This is presumably because the eggs were not soaked with black tea solution which contains active compounds so that there are still eggs that stick to each other and it is suspected that egg development is disturbed by a lack of oxygen intake for metabolism. This condition causes less optimal egg development for metabolism and energy in breaking the egg (Prihatini, 2023). Rizki *et al.* (2023) stated that other factors that can cause low hatching rates are eggs that do not develop after being fertilized, changes in the physiological abilities of eggs during embryogenesis. External factors that determine the success of egg hatching include water temperature, pH, dissolved oxygen and so on (Heltonika, 2014). The results of this study showed D0W0 (dose 0 g/L and time 0 minutes) in line with several researchers who showed that the lowest hatching rate was 11% in African catfish eggs (Yustiati *et al.*, 2021), in asian redtail catfish 27.53% (Badarullah *et al.*, 2020).

#### **Effect of Different Doses and Immersion Time on the Survival of Stripped Catfish Larvae (*Pangasionodon hypophthalmus*)**

Based on Table 1 it is known that the survival rate of Stripped catfish larvae ranges from 96.65% to 96.99%, the Analysis of Variance Test (ANOVA) showed that there was no effect of the

dose and immersion time of black tea solution, and the interaction between dose and immersion time on survival. Based on Figure 4, it is known that the interaction effect between doses of black tea solution and different immersion times on the survival of Stripped catfish larvae aged seven days was highest in treatment D10W4 (dose of 10 g/L and immersion time of four minutes) which was 96.99% and the lowest in the D0W0 treatment (control) that is equal to 96.65%. It is suspected that in the D10W4 treatment the larvae were able to support the survival rate and indirectly the black tea solution did not have an effect on the survival of the Stripped catfish larvae. Another factor that affects the growth of Stripped catfish larvae is feeding *Tubifex* sp. is a natural feed that is easily digested by the Stripped catfish larvae so that it can maintain viability of the Stripped catfish larvae.

The difference in survival in each treatment was caused by the size and age of the fish which were still vulnerable to surviving well, external influences, for example during siphoning. In addition, Saputra *et al.* (2014) stated that the survival rate of larvae after hatching was also influenced by the quality of the eggs produced by the parents. The better the egg quality, the hatching rate and larval survival will also increase. This is in line with opinion Mustofa *et al.* (2018) which states that the survival rate of fish is heavily influenced by environmental factors, such as handling and water quality. Incorrect handling can cause stress in fish, so that the health condition of fish decreases and can cause death. Other factors include competition between species, lack of food, predators or parasites, human handling, age of the organism and the ability to adapt to the environment (Badarullah *et al.*, 2020).

#### **Water quality**

Based on the water quality measurement data in Table 3, it can be seen that the water quality used in the maintenance of Stripped catfish larvae during the study was within the optimum range and was able to support the growth and survival of fish larvae. The temperature during the study ranged from 26.7 to 27.9°C. This range is feasible for catfish in accordance with the statement of Yuli *et al.* (2017) the optimal temperature for farming Stripped catfish is 27-32°C.

The water temperature in this study met the requirements for the life of Stripped catfish. Dissolved oxygen (DO) levels during the study were found to be around 4.0-5.2 mg/L classified

as optimal. The dissolved oxygen content needed for catfish life ranges from 3-6 ppm, and that the range of DO for the maintenance of Stripped catfish is >5 mg/L (Noprianto *et al.*, 2022). The pH of the water in this study ranged from 6.5-7.1. According to Extrada *et al.* (2020), which stated that the optimal pH for farming Stripped catfish, from pH 6.7-7.8.

## CONCLUSION

The results showed that the dose and duration of immersion eggs in black tea solution (*Camellia sinensis*) had a significant effect ( $P < 0.05$ ) on adhesion, fertilization value, hatching value, but did not have a significant effect ( $P > 0.05$ ) on the survival of Stripped catfish larvae. The best dose of black tea solution is 10 g/L with four minutes of immersion time, egg adhesion is 15.10%, fertilization rate is 94.13%, hatching rate is 92.88%, and survival rate for seven days of rearing is 96.99%. The development of embryogenesis hatch with a time of 21 hours 30 minutes.

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