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Effect of Soaking Tawes Fish Eggs (*Barbonymus gonionotus*) in Tea Solution (*Camellia sinensis*) on the Hatchability and Survival of Larvae

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Abstract

Tawes fish (Barbonymus gonionotus) is one of the fish with high production demand, but seed production is still low. One of the obstacles is fungal attacks. An alternative to overcome fungal attacks in the hatching phase of fish eggs is to use one of the plants that is anti-fungal. One alternative ingredient that is safe and can be used in controlling fungi is using tea. The active compounds of flavonoids, tannins, saponins found in tea are expected to be natural alternative ingredients for anti-fungals. This study aims to determine the effect and optimum concentration of tea solution (C. sinensis) on the hatching power and survival rate of tawes fish larvae (B. gonionotus). This study was conducted in April 2023 at the UPT Balai Benih Ikan Pandak Banyumas, Central Java. The method used was an experiment with a Completely Randomized Design (CRD) with 4 replications and 5 replications each. The hatching media was added with tea solution (C. sinensis) with concentrations A (0 g/l), B (3 g/l), C (6 g/l), and D (9 g/l). The data observed included the length of egg hatching time, yolk absorption time, egg hatchability (HR), survival rate (SR), and water quality. The results showed that soaking eggs in tea solution affected the hatching power of eggs and the survival of tawes fish larvae. The results of the hatching rate values in succession from treatments A to D were $64.00 \pm 4.18a\%$, $81.00 \pm 4.18c\%$, $85.00 \pm 3.54c\%$ and $71.00 \pm 4.18b\%$, while the results of the survival rate values in succession from treatments A to D were $62.47 \pm 3.15a\%$, $75.41 \pm 9.46bc\%$, $79.90 \pm 3.86c\%$, and $67.56 \pm 6.33d$. The optimum dose of soaking tawes fish eggs in tea leaf solution for the hatching power of eggs and the survival of tawes fish larvae was 4.96 - 4.98 g/l.

Keywords: Barbonymus gonionotus; Camelia sinensis; egg hatchability

ABSTRAK

Ikan tawes (*Barbonymus gonionotus*) merupakan salah satu ikan yang permintaan produksinya tinggi, akan tetapi produksi benih masih rendah. Salah satu kendalanya yaitu serangan jamur. Alternatif untuk mengatasi serangan jamur dalam fase penetasan telur ikan yaitu menggunakan salah satu tanaman yang bersifat anti jamur. Salah satu bahan alternatif yang aman dan dapat digunakan dalam pengendalian jamur yaitu menggunakan teh. Senyawa aktif flavonoid, tanin, saponin yang terdapat pada teh diharapkan mampu menjadi bahan alternatif alami untuk dimanfaatkan sebagai anti jamur. Penelitian ini bertujuan untuk mengetahui pengaruh dan konsentrasi optimum larutan teh (*C. sinensis*) terhadap daya tetas dan tingkat kelulushidupan larva ikan tawes (*B. gonionotus*). Penelitian ini dilaksanakan pada bulan April 2023 di UPT Balai Benih Ikan Pandak Banyumas, Jawa Tengah. Metode yang digunakan adalah eksperimen dengan Rancangan Acak Lengkap (RAL) dengan 4 ulangan dan masing-masing 5 ulangan. Media penetasan ditambahkan larutan teh (*C. sinensis*) dengan konsentrasi A (0 g/l), B

(3 g/l), C (6 g/l), dan D (9 g/l). Data yang diamati meliputi lama waktu penetasan telur, lama waktu penyerapan kuning telur, *hatching rate*, *survival rate* dan kualitas air. Hasil penelitian menunjukkan bahwa perendaman telur dalam larutan teh berpengaruh terhadap daya tetas telur dan kelulushidupan larva ikan tawes. Hasil nilai *hatching rate* berturut-turut dari perlakuan A sampai D yaitu 64,00±4,18^a%, 81,00±4,18^c%, 85,00±3,54^c% dan 71,00±4,18^b%, sedangkan untuk hasil nilai *survival rate* berturut-turut dari perlakuan A sampai D yaitu 62,47±3,15^a%, 75,41±9,46^{bc}%, 79,90±3,86^c%, dan 67,56±6,33^d)%. Dosis optimum perendaman telur ikan tawes dalam larutan daun teh terhadap daya tetas telur dan kelulushidupan larva ikan tawes yaitu 4,96 – 4,98 g/l.

Kata kunci: *Barbonymus gonionotus*; *Camelia sinensis*; daya tetas

INTRODUCTION

Tawes (*Barbonymus gonionotus*) is a freshwater fish species that is quite well-known in Indonesia. Its production increases every year, with total yields reaching 14,048 tons in 2015 and 44,210 tons in 2016 (Directorate General of Aquaculture, 2016). This indicates that the tawes fish farming business has promising prospects. According to Diana and Safutra (2018), tawes fish is one of the economically valuable freshwater fish species and is potential for cultivation because it does not require specially constructed ponds and can be cultivated throughout the year. This fish also has nutritional content per 100 grams, including 19 g of protein, 13 g of fat, 150 mg of phosphorus, 150 IU of vitamin A, and 48 mg of calcium (Nio, 2012 in Kiranawati *et al.*, 2021). Hatchery is an essential part of aquaculture activities. Fish seed is one of the determining factors in efforts to increase aquaculture production (Afriani, 2016). However, in the hatchery process of tawes fish, a common problem is the high mortality rate during egg hatching. According to Agustin and Rahardja (2013), seed production of tawes fish is still relatively low, only producing about 10,000 eggs with a hatching rate of just 22%.

One of the major obstacles in hatching tawes fish eggs is fungal infection. *Saprolegnia sp.* is a type of fungus that commonly infects freshwater fish, including tawes fish (Rahmayanti *et al.*, 2017). Diana *et al.* (2017) reported that tawes fish egg mortality could reach 45.66% due to fungi that initially appear harmless, but if left untreated, will spread to other eggs, causing them to die. The development of the fungus is facilitated by the presence of an oil layer on the eggs, which spreads to viable ones.

One of the plants that can be used as an antifungal agent during the egg hatching phase is tea. Tea is considered a safe and effective alternative material for fungal prevention (Inamdar *et al.*, 2014). Phytochemical screening of tea has shown that it contains secondary metabolites such as alkaloids, saponins, tannins, phenolics, flavonoids, steroids, and glycosides (Martono and Setiyono, 2014; Nugraheni *et al.*, 2022). Tea contains tannins at levels ranging from 9–20% (Rossi, 2010). Tannins exhibit antifungal activity by disrupting fungal growth and inhibiting essential enzymes for their survival (Pelu *et al.*, 2022). Additionally, the saponins in tea are antifungal, acting by damaging fungal cell membranes, which ultimately leads to cell death (Yuliana *et al.*, 2015).

A study by Muhlis *et al.*, (2019) showed that soaking in tea leaf extract at a dose of 0.6 ml/L had a significant effect on the survival rate of catfish, with the best result at 89.27%. A treatment that can be applied to improve hatching rates and larval survival of tawes fish is by soaking the eggs in tea solution at various concentrations. This study aims to determine the effect of different doses of tea solution on the hatching rate and larval survival of tawes.

MATERIALS AND METHODS

This research was conducted at UPT Balai Benih Ikan Pandak, Banyumas, Central Java. The equipment used included spawning tools, egg soaking apparatus, tea solution preparation tools, and water quality measuring instruments.

The method used in this study was an experimental method. The experimental design was a Completely Randomized Design (CRD) with 4 treatments and 5 replications each. The tea leaf solution used was based on research conducted by Baharudin *et al.* (2016), as follows:

- A. Egg soaking with 0 g/L tea dose
- B. Egg soaking with 3 g/L tea dose
- C. Egg soaking with 6 g/L tea dose
- D. Egg soaking with 9 g/L tea dose

The test subjects used in this study were tawes fish eggs (*B. gonionotus*) sourced from UPT Balai Benih Ikan Pandak, Banyumas, Central Java. The broodstock was semi-artificially spawned. The test eggs were fertilized tawes fish eggs. The rearing container used had a 2-liter volume with a density of 20 tawes fish eggs per container. The eggs were soaked in the tea leaf solution for 4 minutes and then transferred to the rearing containers. Embryo development of the tawes fish was observed under a microscope to determine the development phase until the eggs hatched and the hatching time duration.

The method used for preparing the tea solution (*C. sinensis*) referred to Yustiati *et al.* (2021). The procedure involved weighing tea according to the desired treatment doses: 3 g, 6 g, and 9 g. Water was boiled to 100°C. For treatment A, freshwater was used. For treatment B, one liter of boiling water was mixed with 3 grams of tea to

obtain a 3 g/L concentration. For treatments C and D, the same procedure was followed but with 6 g and 9 g of tea, resulting in 6 g/L and 9 g/L concentrations.

Data Collection

Hatching Time

The calculation of the hatching time is performed using the formula proposed by Wahyuningtias *et al.* (2015), as follows:

$$HT = H_t - H_0$$

Keterangan:

HT = Hatching Time (hatching duration) H_t = Final hatching time (hours)

H_0 = Post-fertilization time (hours)

Yolk Absorption Time

The calculation of the yolk absorption time is performed using the formula proposed by Adriana *et al.*, (2013), as follows:

Keterangan:

WPkt = Yolk absorption time

t_{kh} = Time when yolk is exhausted (hours) t_n = Hatching time (hours)

Hatching Rate

$$WPkt = t_{kh} - t_n$$

The hatching rate is calculated using the formula from Tumanung *et al.*, (2015), as follows:

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

Survival Rate

The survival rate of larvae is calculated using the formula from Hidayat *et al.*, (2013), as follows:

$$SR = \frac{\text{number of fish alive at the end of the rearing (fish)}}{\text{number of fish at the start of the rearing (fish)}} \times 100\%$$

Water Quality Parameters

The water quality in this study was measured using a Water Quality Checker. According to Triwardani *et al.*, (2022), the water quality parameters measured during the study include temperature (°C), pH, and dissolved oxygen (DO). Water quality measurements were taken every morning and evening in each of the fish egg hatching containers.

Data Analysis













The data obtained from the study were analyzed statistically. The data were analyzed using normality tests, homogeneity tests, additivity tests, analysis of variance (ANOVA), and Duncan's test. For the data on egg development and water quality, descriptive analysis was used.





























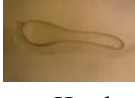

RESULT AND DISCUSSION

Result

Hatching Time

The results of the embryonic development of Tawes fish are presented in Table 1.

Observation	Treatment			
	A (0 g/L)	B (3 g/L)	C (6g/L)	D (9g/L)
0 th minute	 Cleavage	 Cleavage	 Cleavage	 Cleavage
60 th minute	 Morula	 Morula	 Morula	 Morula
120 th minute	 Blastula	 Blastula	 Blastula	 Blastula

180 th minute	 Blastula	 Blastula	 Blastula	 Blastula
240 th minute	 Blastula	 Blastula	 Gastrula	 Gastrula
300 th minute	 Gastrula	 Gastrula	 Gastrula	 Gastrula
360 th minute	 Gastrula	 Organogenesis	 Organogenesis	 Organogenesis
420 th minute	 Organogenesis	 Organogenesis	 Organogenesis	 Organogenesis
480 th minute	 Organogenesis	 Organogenesis	 Organogenesis	 Organogenesis
540 th minute	 Organogenesis	 Organogenesis	 Hatch	 Hatch
600 th minute	 Hatch	 Hatch		

The development of the eggs, when first observed at the 60th minute, showed that treatments A, B, C, and D had already entered the morula stage. At the 120th minute, all treatments were in the blastula stage. The blastula stage lasted 240 minutes for treatments A and B, while treatments C and D required only 180 minutes. Subsequently, the gastrula stage for treatment A lasted 360 minutes, whereas treatments B, C, and D required 300 minutes. Differences in egg development also appeared during the transition from the gastrula stage to organogenesis. Treatments A and B took 540 minutes and hatched at the 600th minute, while treatments C and D required 480 minutes and hatched at the 540th minute. Based on observations and the calculated hatching time of Tawes fish eggs (*Barbonymus gonionotus*), it was found that the eggs in treatments C and D hatched at the 540th minute, which was faster than those in treatments A and B, which hatched at the 600th minute.

Yolk Absorption Time

The observation results of yolk absorption duration in Tawes fish (*Barbonymus gonionotus*) are presented in Figure 1 below:

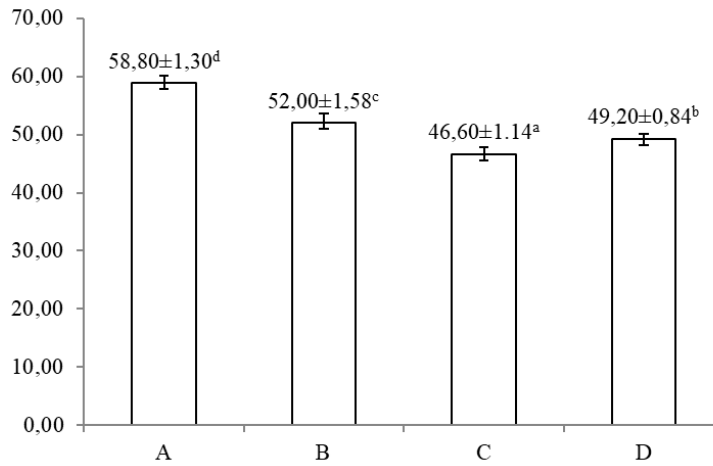


Figure 1. Yolk Absorption Time of Tawes (*B. gonionotus*)

Hatching Rate

The observation results of the hatching rate of Tawes fish (*Barbonymus gonionotus*) are presented in Figure 2 below:

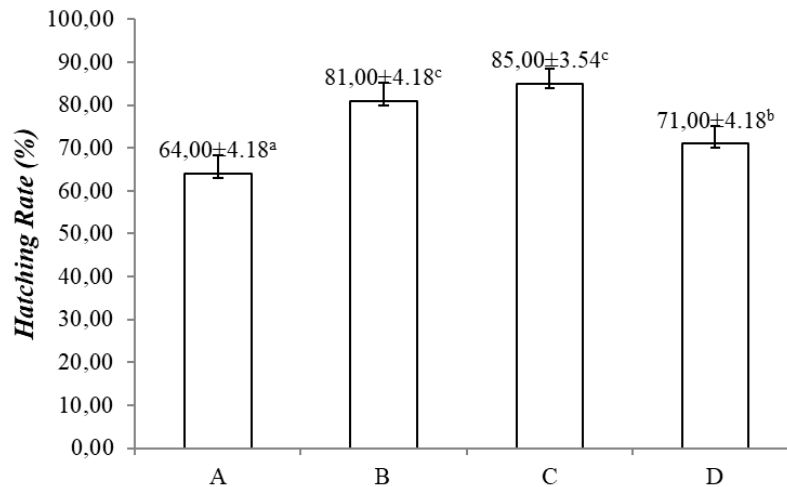


Figure 2. Hatching Rate of Tawes (*B. gonionotus*)

Survival Rate

The observation results of the survival rate of Tawes fish (*Barbonymus gonionotus*) are presented in Figure 3 below:

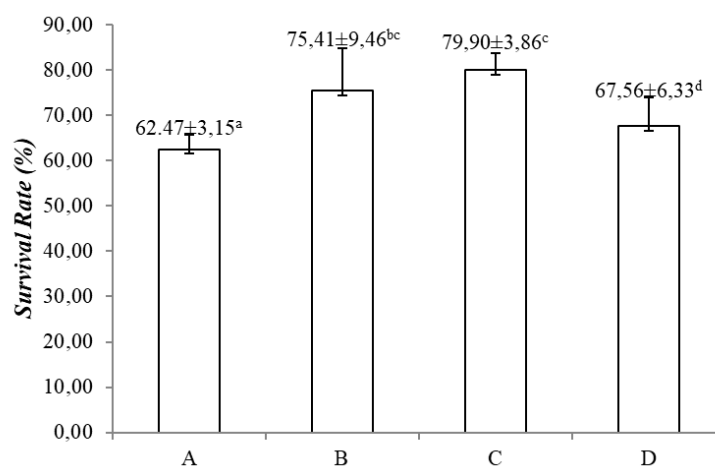


Figure 3. *Survival Rate of Tawes (B. gonionotus)*

Water Quality

The values of water quality parameters, including temperature, pH, and dissolved oxygen (DO), are presented in Table 2.

Treatment	Range of Water Quality Parameter Values		
	Temperature (°C)	DO (mg/l)	pH
A	24,1 – 27,6	8	7,4 – 8,0
B	24,2 – 27,8	8	7,5 – 7,9
C	24,1 – 28,2	8	7,5 – 8,0
D	24,0 – 27,9	8	7,2- 8,0
Feasibility Value	20 - 33°C (Aprilia <i>et al.</i> , 2017)	≥ 4 mg/l (Aprilia <i>et al.</i> , 2017)	6,7 – 8,6 (Aprilia <i>et al.</i> , 2017)

DISCUSSION

Hatching Time

The results of the observation of the hatching time of Tawes fish (*Barbonymus gonionotus*) eggs showed that the fastest hatching occurred in treatments C and D with a hatching time of 9 hours, followed by treatments A and B, which took 10 hours. The organogenesis phase was the longest phase before the larvae hatched, and from the observations, all treatments entered the organogenesis phase at the 360th or 420th minute. The hatching times for each treatment, from treatment A to D, were 10 hours, 10 hours, 9 hours, and 9 hours, respectively. According to Zuraidah and Silkhairi (2016), Tawes fish eggs begin to hatch 9 to 10 hours after fertilization. The hatching percentage increases from 10 to 13 hours. The hatching time of Tawes fish eggs (*B. gonionotus*) is considered relatively short, ranging from 540 to 600 minutes. The hatching time is suspected to be influenced by the eggs' resistance to pathogen attacks that cause egg diseases. Fungal attacks on the eggs can slow down the development process and even cause the eggs to die. This is supported by Diana *et al.* (2017), who stated that initially, fungi attack the fish without causing harm, but if the attack is not stopped, the fungus will spread to other eggs, causing them to die.

The hatching time is also influenced by other factors, such as temperature and the environment. Temperature affects enzymatic processes within the eggs, allowing embryos to develop faster at higher temperatures. The hatching time is also influenced by the activity level of the embryo inside the egg. The more active the embryo moves, the faster the eggs will hatch. Cahyanti *et al.* (2022) noted that Tawes fish eggs hatch after 11 hours and 45 minutes at a temperature of around 28°C. This is relatively fast because Tawes is a small to medium-sized fish species. According to Sukendi (2003) in Nawir *et al.* (2016), egg hatching occurs faster at higher temperatures because metabolic processes happen more quickly at higher temperatures, leading to faster embryo development. The embryo's movements within the egg also become more intensive, resulting in quicker hatching. The differences in hatching time are caused by the embryo's low ability to break free from the eggshell and the increase in adrenaline during hatching, which causes physical stress on the embryo as it attempts to leave the eggshell. The hatching time is also influenced by the pH level of the hatching medium. Like temperature, the pH level can affect the activity of the chorionase enzyme, which plays a role in hatching. An optimum pH condition can accelerate egg hatching. According to Tang and Affandi (2001) in Altiara *et al.* (2016), at a pH of 7.1–9.6, the chorionase enzyme, produced by the endodermal glands in the pharynx region of the embryo, optimally reduces the chorion, which consists of pseudokeratin, making it softer. The embryo becomes more active in movement when hatching is about to occur. These movements are accompanied by faster circular body movements, which accelerate the breakdown of the eggshell, shortening the hatching time.

Yolk Absorption Time

Based on the results of the research conducted, it was found that soaking Tawes fish eggs in tea solution significantly affected the hatching rate of the eggs ($P < 0.05$), with the fastest yolk absorption time observed in treatment C, which was (46.60±1.14a) hours. This was followed by treatment D (49.20±0.84b) hours, treatment B (52.00±1.58c) hours, and the longest yolk absorption time in treatment A (58.80±1.30d) hours. Treatments B, C, and D, which were soaked in tea solution, were generally faster, likely because the yolk had already been used as an energy source for the development of the Tawes fish larvae until they hatched. This is supported by Anpe *et al.* (2017), who stated that the yolk in fish starts to function and is used for the development process beginning 5 hours after fertilization and ends 7 hours after fertilization. In the final stage of development, the energy required by the egg increases to prepare for the hatching process. In the study by Arif *et al.* (2023), the yolk absorption time for

Tawes fish eggs was 54 hours.

The differences in yolk absorption times are thought to be influenced by the development and growth process of each larva observed. Faster yolk absorption is caused by the organogenesis process in the larvae, which requires more energy. According to Herjayanto *et al.* (2017), the energy used during the embryogenesis process comes from the yolk, which is marked by the decreasing size of the yolk as development progresses. According to Safrizal *et al.* (2020), the size of the egg plays a role in the survival of the fish and the yolk absorption time. Larger eggs with higher yolk content contribute to better resistance and higher chances of survival compared to smaller eggs. Yolk absorption during embryogenesis is also influenced by temperature. At optimal temperatures, the efficiency of yolk utilization for tissue formation is maximized. As the ambient temperature increases, the larvae absorb yolk more quickly due to increased metabolic processes (Herjayanto *et al.*, 2017). Additionally, yolk absorption is used for larval metabolism, so once the yolk is exhausted, the larvae will start consuming food available in the water, such as plankton (Mulyani *et al.*, 2015).

Hatching Rate

Based on the results of the research conducted, it was found that soaking Tawes fish eggs in tea solution significantly affected the hatching rate of the eggs ($P < 0.05$). Treatment A (0 g/L) had the lowest hatching rate at (64.00±4.18a)%, while treatments B and C showed the same hatching rate values, with treatment C (6 g/L) at (85.00±3.54c)% and treatment B (3 g/L) at (81.00±4.18c)%. Treatment D (9 g/L) had a hatching rate of (71.00±4.18b)%. The Polynomial Orthogonal test showed a quadratic relationship ($Y = -0.8611x^2 + 8.5833x + 63.75$) with $R^2 = 0.836$. The optimal point was found in treatment C (6 g/L), where the optimum tea solution dosage, derived from this equation, was 4.98 g/L, resulting in a hatching rate of 85.14%. The R^2 value indicates that 83.6% of the hatching rate is influenced by the tea solution, while 16.4% is influenced by other factors not yet identified. The tea solution (*C. sinensis*) affected the hatching rate of the eggs because it contains secondary metabolites such as tannins and flavonoids, which help protect the eggs from fungal attacks that can infect the eggs and cause them to fail to hatch (Malik and Inriyani, 2015; Muhlis *et al.*, 2016). Therefore, the eggs are protected by the antifungal compounds in the tea solution, preventing fungi from easily infecting the Tawes fish eggs. According to Fitri (2007), in the absence of antifungal compounds, the eggs' resistance to fungal attacks would only rely on the strength of the chorion. Fungi that attach to the fish eggs weaken the chorion, making it easier for the fungi to attack and infect the eggs.

The variation in the hatching rate of the eggs is thought to be due to the use of different tea solution dosages. Eggs infected with fungi allow fungal hyphae to grow and penetrate the chorion layer. The fungi absorb nutrients from the eggs, preventing further development, which eventually leads to egg death (Rosidah *et al.*, 2017). Soaking eggs in tea solution at higher dosages can speed up hatching, but if the dosage is too high, it can be detrimental to the eggs, especially if they are still weak and unable to develop, leading to egg death. According to Hasan *et al.* (2016), if the tea solution dosage is too high, the secondary metabolite compounds also increase, causing the eggs to absorb these compounds excessively, which can become toxic and kill the eggs. High concentrations of solutions containing phenolic compounds and tannins not only prevent fungal growth but can also damage the egg tissues and hinder respiration, ultimately leading to egg death (Zuraidah and Silkhairi, 2016).

Survival Rate

Based on the results of the study, it was found that soaking Tawes fish eggs in tea solution had a significant effect on the hatching rate of the eggs ($P < 0.05$). The highest hatching rate was observed in treatment C (6 g/L) with a rate of (79.90±3.86c)%, followed by treatment B (3 g/L) at (75.41±9.46bc)%, treatment D (9 g/L) at (67.56±6.33d)%, and the lowest survival rate in treatment A (0 g/L) at (62.47±3.15a)%. The Polynomial Orthogonal test showed a quadratic relationship ($Y = -0.7022x^2 + 6.9787x + 62.053$) with $R^2 = 0.5851$. The optimal point was found in treatment C (6 g/L), where the optimum tea solution dosage, derived from this equation, was 4.96 g/L, which resulted in a survival rate of 79.39%. The R^2 value indicates that 58.5% of the survival rate is influenced by the tea solution, while 41.5% is influenced by other factors that have not yet been identified. This result suggests that the flavonoids, tannins, and saponins contained in the tea solution provide protection for the fish eggs, preventing fungal attacks. Therefore, the number of viable larvae produced is also quite good. A higher hatching rate indirectly contributes to a higher survival rate of the Tawes fish larvae. According to Gusrina (2008), the number of larvae that survive at the end of the rearing period is closely linked to the hatching rate. The quality of the eggs is a key factor in producing viable larvae. The increase in immunity in both larvae and adults is partly due to the presence of antimicrobial compounds, which improve the success of egg hatching and reduce larval abnormalities (Haser *et al.*, 2018). Salam and Daesusi (2019) also noted that flavonoids and saponins have immunostimulant effects that enhance the body's defense system and prevent disease.

The results also show that the survival rate of the Tawes fish larvae remains relatively low. Several factors affect larval survival, including the quality of the rearing medium and the availability of food. In a study by Winata *et al.* (2018), Tawes fish larvae fed natural food had a survival rate of 85.00%, while Pratama (2019) reported a survival rate of 99.3%. According to Ariyanto *et al.* (2008), low survival rates may occur when the larva's basic

needs, including food and optimal environmental conditions, are not met. After hatching, the larvae rely on food sources for energy to grow. Poor food quality can disrupt larval development, leading to mortality. Additionally, good water quality significantly affects the survival and growth of the fish (Salam and Daesusi, 2019)

Water Quality

Based on the results of the water quality measurements taken over 14 days, the water temperature ranged from 24.0°C to 28.2°C. This temperature range is still considered suitable for supporting the life and growth of Tawes fish larvae, as reinforced by the Indonesian National Standard (SNI) (1999), which states that the optimal temperature for fish larvae production is between 25°C and 30°C. According to Diana and Eri (2018), the normal temperature for hatching Tawes fish eggs ranges from 24°C to 32°C. The dissolved oxygen (DO) level during the study was measured at 8 mg/L. One of the key factors influencing the success of fish egg hatching is the level of dissolved oxygen (Muslim and Danang, 2017). Mahendra (2018) mentioned that fish can survive in water with an oxygen content of 3 mg/L, but to increase fish productivity, the dissolved oxygen level should be above 5 mg/L. The optimal range for dissolved oxygen (DO) for Tawes fish (*P. javanicus*) is above 4 mg/L, as noted by Apriliana *et al.* (2017). The pH level of the water during the study ranged from 7.2 to 8.0. This pH level is considered suitable for the hatching of Tawes fish eggs. If the pH is too low or too high, it can lead to growth disturbances in the fish. According to Yumame *et al.* (2013), a water pH between 6.5 and 9 is still considered suitable for fish farming.

CONCLUSION AND SUGGESTIONS

Conclusion

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

1. Soaking Tawes fish eggs in tea solution (*C. sinensis*) with different doses has a significant effect on hatchability and larval survival.
2. The optimal dose of tea solution for improving egg hatchability and larval survival in Tawes fish (*B. gonionotus*) is between 4.96 and 4.98 g/L.

Suggestions

Based on the research conducted, the following recommendations can be made:

1. It is advisable to conduct a phytochemical test to confirm the content of tannins, saponins, and flavonoids in the tea solution (*C. sinensis*).
2. Soaking eggs in tea solution (*C. sinensis*) with a dose of 4.96-4.98 g/L is recommended as the optimal dose to improve egg hatchability and larval survival of Tawes fish (*B. gonionotus*)

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