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The incubation of Tinfoil barb (*Barbonymus schwanenfeldii*) eggs using funnel system at different temperatures

Siska Mellisa[1,*], Rizky Danang Ramadhan[1], Iwan Hasri[2], Nurfadillah Nurfadillah[1]

[1]*Department of Aquaculture, Faculty of Marine and Fisheries, Universitas Syiah Kuala, Banda Aceh 23111, Indonesia.*
[2]*Department of Aquaculture, Universitas Gajah Putih, Takengon 24552, Indonesia.*

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ABSTRACT

Tinfoil barb (*Barbonymus schwanenfeldii*) is freshwater fish found in Borneo, Java, and Sumatera including in Aceh Province waters. It is known as a native species in Indonesia, locally known as *Tengadak* or *Lampan* and this species is popular for consumption due to its taste. Tinfoil barb is targeted species of inland water for fisheries production. In recent years, the fish population has declined because of overexploitation and habitat degradation. Therefore, this study aimed to analyze the effects of temperature on Tinfoil barb (*B. schwanenfeldii*) eggs incubation using a funnel system. It was conducted at Balai Benih Ikan (BBI) Lukup Badak, Central Aceh Regency. The Completely Randomized Design was used as a statistical analysis method with four treatments and three replications at 22±1 °C, 25±1 °C, 28±1 °C, and 31±1 °C. Hatching was carried out using a funnel system with 100 grain/liter of eggs density for incubation. The parameters measured were hatching time and rate, egg yolk absorption time, the survival rate of the embryo, larvae abnormality, and water quality. The ANOVA test results showed that temperature significantly affected hatching time and rate, egg yolk absorption time, and larvae abnormality ($P<0.05$). In contrast, the effect was insignificant on the survival rate of an embryo, with $P>0.05$. It is concluded that the optimum temperatures for Tinfoil barb eggs incubation were about 25±1 °C–31±1 °C. A funnel system was more effective for incubation than the conventional system.

Introduction

Tinfoil barb (*Barbonymus schwanenfeldii*) is freshwater fish found in Borneo, Java, and Sumatra (Kusmini *et al.,* 2021) including in Aceh Province waters (Muchlisin *et al*., 2015). Tinfoil barb is one of the native fish species in Indonesia, locally known as *Tengadak* or *Lampan* and this species is popular for consumption due to its taste (Kusmini *et al.,* 2018; 2020; 2021). Tinfoil barb is targeted species of inland water for fisheries production. In recent years, the fish population has declined because of overexploitation and habitat degradation (Huwoyon *et al*., 2010). Kusmini *et al.* (2021) stated that the domestication of Tinfoil barb is a key strategy for diversification of aquaculture production. The Tinfoil barb culture has been developed to domestication stage at Balai Benih Ikan (BBI) Lukup Badak, Aceh Tengah regency. However, the development faces obstacles in the breeding process due to the low percentage of egg hatching during the incubation. The method and incubation temperature of Tinfoil barb eggs were assumed not effective. Therefore, it was important to examine the method and incubation temperature of Tinfoil barb eggs.

One method used for eggs incubation was the funnel system. The incubator, also known as *Zoug Jar,* was used for a long time as an incubation tool in almost all types of fish. The incubation mechanism involves water flow from the bottom to the top of the incubator (Rustadi, 2002). The water flow creates turbulence and oxygenation that stimulate eggs to keep moving (Prakoso *et al*., 2018). The study of fish egg incubation using a funnel system has been applied on incubation egg of several fishes, for example *Osteochilus kappenii*

* Corresponding author.
Email address: siska_mellisa@unsyiah.ac.id

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(Ulyana *et al*., 2018), *Hemibagrus nemurus* (Prakoso *et al*., 2018) and *Pangasius djambal* (Slembrouck *et al*., 2005).

The temperature and method used were substantial in egg incubation. The different temperatures significantly affected embryo development. Inappropriate temperatures disturb the embryogenesis process and impact the hatching rate, resulting in larvae abnormalities (Kupren *et al*., 2008; Naskuroh *et al*., 2018; Rahmadi *et al*., 2022). Appropriate temperatures significantly affected the incubation period, hatching rate, normality, and survival rate of the larvae produced (Muslim *et al*., 2018; Sugiarto *et al*., 2015; Safraini *et al*., 2019; Nur *et al*., 2020). The effect of temperature on the time of incubation eggs were reported for some species, including *Anabas testudineus* (Putri *et al*., 2013), *Barbonymus gonionotus* (Naskuroh *et al*., 2018), *Channa striata* (Muslim *et al*., 2018), *Hellostoma temminckii* (Wahyuningtias *et al*., 2015), *Osteochilus melanopleura* (Rusila *et al*., 2017), *Osteochilus hasselti* (Hutagalung *et al*., 2018), and *Osphronemus gourami* (Pratama *et al*., 2018). However, no study has been conducted on *B. schwanenfeldii.* Therefore, this study aimed to determine the best water temperature for incubation time of Tinfoil barb (*B. schwanenfeldii*) eggs using the funnel system.

Materials and Methods

Site and time

This study was conducted at Balai Benih Ikan (BBI) Lukup Badak, Central Aceh Regency. The tools and materials used included a heater, pH meters, an aerator, a 0.5-liter plastic bottle, a water pump, a PVC pipe, a microscope, and Tinfoil barb eggs. The preliminary experiment used Completely Randomized Design as a statistical analysis method with four treatments and three replications of treatments A, B, C, and D at 22±1 °C, 25±1 °C, 28±1 °C, and 31±1 °C, respectively.

Four containers with 60 liters of water as incubation source and 12 funnel plastic bottles of 0.5 liters as incubators were used. Each container had 3 incubators inside and was modified with an output waters duct on the side. The holder pole was used to put the incubator in the containers.

The incubation used mountain water filled in containers and soaked for 24 hours. The containers were set with a heater, water pump, and aerator. The water in the container was flowed to the incubator by the water pump connected with a PVC pipe as a duct. The egg samples collected from broodstocks of Tinfoil barb at BBI Lukup Badak had been spawned semi-artificially. All the eggs had been fertilized and randomly collected with 100 grains/liter eggs density. The samples were put in an incubator with normal temperature and increased slowly to treatment temperature. Moreover, the water quality was controlled daily by filtering from waste residues such as dead eggs, larvae, and other disturbing particles in incubation.

Parameters

Hatching time

The hatching time is the period needed by eggs to hatch and counted using the following formula by Effendie (1997):

$$HT = Ht - Ho$$

Where HT is the hatching time in minutes, Ht is the end time of hatching, and Ho is the time after the egg fertilization.

Hatching rate

The hatching rate is the percentage amount of larvae produced by egg incubation and counted using the following formulas by Effendie (2002):

$$\text{Hatching Rate (\%)} = \frac{Total\ hatched\ eggs}{Total\ eggs} \times 100$$

Egg yolk absorption

The egg yolk absorption is the time needed by larvae to absorb the yolk from pre-larva until it is empty. It is counted using the following formula by Andriana *et al.* (2013):

Egg yolk absorption = the yolk time end (minutes) - the time of hatched egg (minutes)

The survival rate of embryo

An embryo's survival rate is the percentage of total larva survive from incubation until the end of the study. It is counted using the following formula by Muchlisin *et al.* (2016):

$$\text{Survival Rate (\%)} = \frac{(No - Nt)}{No} \times 100$$

Where, Nt is total larvae at the end of the study, and No is total larvae at the start of experiment.

Larvae abnormality

Larvae abnormality is the percentage amount of larva hatched abnormally. It is counted using the following formula by Wirawan (2005):

$$\text{Larvae abnormality (\%)} = \frac{Total\ abnormal\ larvae}{Total\ hatched\ egg} \times 100$$

Data analysis

Data were processed with the Analysis of Variance (ANOVA) method on parameters of hatching time and rate, egg yolk absorption, larvae abnormality, and survival rate of an embryo. When the data results were significant with $P<0.05$), they were considered valid for a further test by coefficient variety.

Results

The hatching time and rate, egg yolk absorption time, larvae abnormality, and embryo survival rate were about 1963.33-3177.33 minutes, 78%-94.66%, 2963-4602.66 minutes, 0%-14.54%, and 71.27%-83.09%, respectively (Table 1). The ANOVA test showed that different temperatures gave a significant effect on the incubation time of Tinfoil barb eggs ($P<0.05$). The effects were seen on the hatching time and rate, egg yolk absorption time, and larva abnormality. However, there was no significant effect on the survival rate of an embryo. The further test showed that B, C, and D treatments were not significantly different, while the A treatment was significantly different from those three.

The relationship between temperature and egg yolk absorption time was shown on Figure 1. It was shown that every one unit increase in temperature can cause a decrease in egg yolk absorption time by one unit. The relationship was explained by equation $y = 8239.5 – 173.54x$ with correlation value 92.11%.

Discussion

The incubation of Tinfoil barb (*B. schwanenfeldii*) fish eggs at different temperatures showed variations in hatching times caused by different treatments. According to Melianawati *et al.* (2010); Sugiarto *et al.* (2015), higher temperatures increase the hatching rate. Yamagami (1998) also found that increasing incubation temperature accelerates the hatching enzyme secretion. As a result, the hatching process is quicker at higher than at low temperatures.

Table 1. The results of ANOVA test on incubation of Tinfoil barb eggs (*B. schwanenfeldii*) using funnel system at different temperatures

Treatment	Hatching time (minutes)	Hatching rate (%)	Egg yolk absorption time (minutes)	Larva Abnormality (%)	SRE (%)
A	3177.33±7.02^{b}	78.00±10.00^{a}	4602.66±29.14^{d}	0.00^{a}	74.43±10.82^{a}
B	2029.66±21.96^{a}	94.66±1.15^{b}	3642.00±37.58^{c}	2.50±4.33^{a}	83.09±2.13^{a}
C	2013.33±16.28^{a}	93.33±4.61^{b}	3354.66±23.62^{b}	6.64±4.74^{ab}	71.27±19.4^{a}
D	1963.33±68.39^{a}	93.33±4.62^{b}	2963.00±67.55^{a}	14.54±7.92^{b}	77.46±12.12^{a}

Note: The different superscript codes in the same column showed significant differences ($p<0.05$).

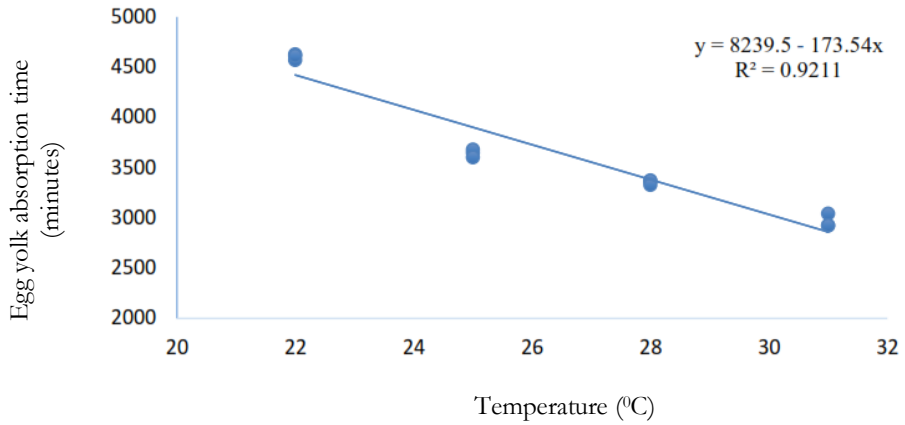

Figure 1. The relationship between temperature and egg yolk absorption time of Tinfoil barb

The hatching time of Tinfoil barb was slower than the hatching time for other species. Research conducted by Wahyuningtias *et al.* (2015) on tambakan fish (*H. temminckii*), showed that the hatching time ranged from 874.4 minutes to 1203.6 minutes at the temperature range of 24–31 °C. Putri *et al* (2013) repored that the fastest hatching time of *Anabas testudineus* was 964 minutes at 34 °C, but Rahmadi *et al.* (2021) reported the temperature was 1118 minutes at 28 °C. Naskuroh *et al.* (2018) revealed that the hatching time of *tawes* fish (*B. gonionotus*) ranged from 778.2 minutes to 820.2 minutes at the temperature range of 25–28 °C. In contrast, Cahyanti *et al.* (2021) reported that the hatching time of Tinfoil barb was 640 minutes at 28°C.

The hatching time in incubation was influenced by water temperatures and embryo activities (Putri *et al.* 2013). According to Ulyana *et al.* (2018),

embryo activities and *chorionase* secretion were influenced by internal and external factors such as temperature, oxygen, pH, salinity, and light intensity. Incubation using a funnel system caused the eggs to keep moving, making the embryo more active. Sukendi (2005) stated that the more active the embryo, the quicker the hatching time.

The funnel system is a hatching method used for effective egg incubation. Pandit *et al.* (2017) stated that hatching rate using a funnel system produced higher hatching rate compared to hatching using an aquarium (conventional system) on tilapia (*Oreochromis niloticus*). Moreover, it has been reported that the hatching rate of *Hemibagrus nemurus* in the funnel system (65.79±5.49%) was higher than in the tray system (30.60±1.91%) (Prakoso *et al.*, 2018). According to Rustadi (2002), the funnel system recirculates water to the bottom of the funnel, creating turbulence and facilitating egg movement. The active egg movement makes the eggs hatch quicker than a passive movement.

The results showed that incubating Tinfoil barb eggs using a funnel system at different temperatures increased the hatching rate from 78.00±10.00% to 94.6±1.15%. The higher percentage hatching rate was assumably caused by the use of a funnel system that indirectly controlled the quality of water and eggs also because of high temperature. The funnel system was more effective for incubating Tinfoil barb eggs than the conventional system (Prakoso *et al.*, 2018; Pandit *et al.*, 2017). Study by Naskuroh *et al.* (2018) revealed that temperature has an important role in hatching eggs, fast or slow the process of hatching eggs depends on the temperature of the surrounding water. In contrast, Putri *et al.* (2013); Pratama *et al.* (2018) found that temperature did not affect the hatching rate but it can be affected the hatching time of eggs. In addition, Sukendi (2003) stated that the hatching of eggs will be faster at high temperatures because at the optimum temperature, the metabolism process will occur faster so that the development of embryo will be fast then the movement of the embryo in the shell will more intensive so that hatching is faster than usually. Several studies found that the hatching rate of Tinfoil barb (*B. schwanenfeldii*) was 73.67-76.41% (Kusmini *et al.*, 2020). The wild broodstocks from Borneo and Java had hatching rates of 77.44±1.03% and 73.67±3.15%, respectively (Kusmini *et al.*, 2016). Hatching rate of *Barbonymus gonionotus* was found at temperature 26^{0}C about 93% (Naskuroh *et al.*, 2018). The highest hatching rate in this study was 94.6±1.15% at 25^{0}C. Compared to other species, *Osphronemus gouramy* has a hatching rate of 98.83±0.76% at 32^{0}C (Pratama *et al.*, 2018), hatching rate of *Channa striata* was 86.3% at 28±0.5 ^{0}C (Muslim *et al.*, 2018), hatching rate of *Hellostoma temminckii* was 78% at 26-28 ^{0}C (Wahyuningtias *et al.*, 2015), *Plectropoma laevis* has a hatching rate of 92.25% at 30 ^{0}C (Andriyanto *et al.*, 2013). Differences in hatching rate between species due to the different response of each fish to changes in temperature of incubation.

Temperature influences the hatching process. Tang and Affandi (2002; 2017) found that very high or low temperatures disturb the incubation process. Extreme temperatures kill the embryo and halt the incubation process. According to Hutagalung (2016), embryo metabolism works quicker at high temperatures that accelerate egg development but also disturb the hatching process and cause death.

The result showed that egg yolk absorption time on Tinfoil barb was significantly different between treatments. In this study, the fastest egg yolk absoption time was found at the highest temperature (31±1 ^{o}C) about 2963.00±67.55 minutes. Different egg yolk absorption time values can be caused by different larvae metabolic rates between treatments. According to Wahyuningtias *et al.* (2015), the increase of egg yolk absorption rate due to activity metabolism affected by incubation temperature treatment. Putri *et al.* (2013) revealed that if the temperature is high, the body's metabolic will faster and vice versa. In addition, high temperatures influence larva metabolism in order to egg yolk absorption will be faster than low temperatures (Muslim *et al.*, 2018; Mulyani *et al.*, 2015). In line with the study of Wahyuningtias *et al.* (2015), high temperatures resulting in fast egg yolk absorption time of *Hellostoma temminckii,* its about 68.79 hours at 29-31^{0}C. Mulyani *et al.* (2015) mentioned that incubation eggs of *Osteoglossum bicirrhosum* at 30^{0}C and 32^{0}C has a fastest egg yolk absorption time. Pandit *et al.* (2017) found that egg yolk absorption time of *Orechromis niloticus* was 140 hours.

Based on Figure 1, we can see there was a close relationship between temperature and egg yolk absorption time which is expressed by the regression equation y = 8239.5 – 173.54x. This equation can be interpreted that every 1^{0}C rise in temperature will reduce egg yolk absorption time by 173.54 minutes. An increase in temperature causes metabolic process of the larvae will increase at high temperatures so that the egg yolk absorption rate is faster than normal conditions. According to the statement of Lam *et al.* (2006), a change in temperature of 1 ^{0}C will affect changes in the body's metabolic reactions by 10%.

High temperatures increase the larvae's ability to obtain oxygen, while low temperatures make them

passive. Increased larvae activity increases the energy used from egg yolk. In line with this, Budiardi *et al.* (2005) stated that the egg yolk energy is needed when the incubation temperature is high to increase the yolk absorption rate. The larvae's activity and yolk absorption rate are slowed under low incubation temperatures.

Abnormality is the deformity of the head, body, and tail (Wirawan, 2005). The abnormalities in fish larvae causes the organ the body cannot develop properly. Andriyanto *et al.* (2013) stated that abnormalities that occur in each species of fish larvae cause fish organs cannot grow perfectly. In addition, abnormalities can be determined from the shape of body be it a curved tail or the bony part of the body that curves downward (Nurlian *et al.*, 2020). In this study, larvae abnormalities occurred at 25°C, 28°C, and 31°C, but not at 22 °C. The highest abnormalities value was 14.54±7.92 found at 31°C. Cahyanti *et al.* (2021) found the abnormalities value of Tinfoil barb was 16% at 28°C. It was suspected that the incubation temperature caused the larvae abnormality and this supports Muslim *et al.* (2018). Casenave *et al.* (2013) revealed that an increase in temperature causes anomalies in an embryo's nerve development. Therefore, higher incubation temperatures increase the risk of abnormality. In contrast, Wahyuningtias *et al.* (2015) found that different temperatures of egg incubation did not affect abnormality of *Hellostoma temminckii* larvae.

Agatha *et al.* (2021) states that the fertilized egg will develop and hatch normally if supported by good environment condition, including oxygen enough, the suitable temperature and microorganisms free. Embryo has a temperature tolerance limit in development process (Yuliani *et al.* 2020). In addition, the hatching phase of eggs is a phase that is susceptible to environmental influences, especially temperature. According to Mukti (2005), temperatures that are too high can cause the activity of hatching enzymes to be disturbed and can produce abnormal larvae. The low abnormality value in the control treatment could be due to the slower hatching process so that the energy available would be focused on embryonic development rather than for movement in the shell.

According to Effendi (2004), fish survival is the percentage of fish that live from the beginning to the end of the maintenance. Measurement of survival parameters was observed after the egg yolk of the larvae was utterly depleted. The best treatment for the survival of Tinfoil barb larvae was found at a temperature of 25±1 ^{0}C which indicated that the temperature was optimal for the life of Tinfoil barb larvae. Budiardi *et al.* (2005) reported that embryos which are incubated at optimal temperatures will increase their resistance larvae produced so that their survival increases.

Rusila *et al.* (2017) stated that the death of larvae could be caused by the inability to adapt to fluctuations in water temperature. According to Laurel *et al.* (2008), temperature influences the size and survival rate of larvae embryology development and metabolism after hatching. Unfriendly environmental conditions such as very high or low temperatures and direct light penetration might cause larvae's death, specifically in the transition or critical phase.

Conclusion

This study shows that the optimum temperatures for Tinfoil barb eggs incubation were about 25±1^{0}C -31±1^{0}C. In addition, the funnel system was more effective than a conventional system for egg incubation.

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