

Effect of Different pH on Embryogenesis and Hatching Rate of Srikandi Strain Tilapia Eggs (*Oreochromis aureus* X *Oreochromis niloticus*) in Incubator

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Abstract

Tilapia (*Oreochromis niloticus*) is one of the freshwater fish species which is a leading commodity in the aquaculture sector. The use of an incubator in hatching fish eggs can produce seeds with relatively the same age, the environment is more controlled so it will be easier to manipulate environmental factors that can affect hatching eggs to produce seeds of superior quality. This study aims to determine the description of embryogenesis and hatching rate of Srikandi strain tilapia eggs hatched at different pH. The experimental design used was a completely randomized design (CRD). The treatment consisted of four treatments, namely A (pH 6), B (pH 7), C (pH 8) and D (pH 9) with four replications. Statistical analysis used ANOVA (Analysis of Variance) and to find out the difference between one treatment and another, Duncan's Multiple Range Test was performed. The results showed that different pH treatments had an effect on the embryogenesis of Srikandi tilapia and also had a very significant effect on the hatchability of Srikandi tilapia eggs. The average hatchability of Srikandi tilapia eggs was highest in treatment B (pH 7) of 87.5% although it was not significantly different from treatment C (pH 8) of 80%.

Keywords: Egg hatchability, Embryogenesis, pH, Srikandi strain Tilapia.

1. Introduction

Tilapia (*Oreochromis niloticus*) is one of the freshwater fish species which is a leading commodity in the aquaculture sector. Tilapia is a commodity that is in demand by the people of Indonesia which causes market demand to increase and tilapia is also an export commodity to the United States in the form of fillets (Directorate General of Aquaculture, 2019). Tilapia production in Indonesia has always increased, recorded in 2016 the production of tilapia reached 1,114,156 tons, in 2017 it was 1,265,201 tons and in the third quarter of 2018 it reached 579,688 tons (Directorate General of Aquaculture, 2019).

Tilapia strain Srikandi is a superior strain of tilapia which was released by the Minister of Maritime Affairs and Fisheries in 2012. Srikandi tilapia is a hybrid fish that tolerates high salinity (10-30 ppt) obtained by crossing selected female nirvana tilapia. *Oreochromis niloticus* with male *Oreochromis aureus* (Hadie et al, 2013). Tilapia strain Srikandi has a competitive advantage because it has the best growth performance at a salinity of 10-30 ppt compared to other tilapia strains. Therefore, the development of Srikandi tilapia strains in aquaculture areas is a strategic step that can boost fishery production (Hadie et al., 2011).

Cultivation is one way to meet the increasing demand for tilapia. Hatchery is one of the stages in aquaculture, where from the development of fish embryos to the process of hatching, it becomes one of the periods that can increase mass mortality. Water quality is one of the factors that affect cultivation activities. A pH value that is too acidic or too alkaline can affect the embryogenesis of

fish eggs and the hatchability of fish eggs. Hatchery which is included in aquaculture activities is a job that has the aim of producing fish seeds of up to a certain size. The hatchery process begins with the maintenance of brood fish, the spawning process takes place, care for fish eggs until they hatch, care for newly hatched seeds, then care for the seeds until a certain size (Mutalib and Tunggal, 2017). The early period of embryo development until the hatching of fish eggs is said to increase mass mortality (Andriyanto et al., 2013).

Water quality in aquaculture activities greatly influences fish growth., Optimal pH is one of the factors for the success of fish farming activities. According to (Dewanti, 2017), a pH value below 7 can cause fertilized fish eggs to experience disturbances in the embryogenesis process. A pH above 7 also affects the process of embryogenesis of fish eggs because the lime contained in the waters will make the walls of the chorion harder and the substances needed in the process of embryogenesis cannot enter.

The use of an incubator in hatching fish eggs can produce seeds of relatively the same age, the environment is more controlled so it will be easier to manipulate environmental factors that can affect hatching of eggs to produce seeds with superior quality (Aanand and Rajeswari, 2018).

Wardani's research (2017) conducted at different pH had a very significant different effect on the hatching speed of Baung fish (*Mystus nemurus*), egg hatchability and larval survival. As well as significantly different effect on abnormalities. The fastest egg hatching time was obtained at pH 8 with a hatching time of 29 hours 47 minutes. The highest percentage of hatchability was obtained at pH 9 of 82.22% and the lowest larval abnormality value was obtained at pH 9 of 4.48%, while the highest percentage of survival was at pH 9 of 79.36%.

Information regarding embryogenesis development and hatchability of the Srikandi tilapia strain is urgently needed as a step to overcome problems in the Srikandi tilapia hatchery activities. In tilapia harvesting activities, water quality such as pH is very influential in the process of embryogenesis and hatchability of tilapia eggs. Therefore, it is necessary to conduct research on the effect of different pH on embryogenesis and hatchability of the Srikandi tilapia strain (*Oreochromis aureus* x *Oreochromis niloticus*) in an incubator.

METHODS

Time and Location of Research

This research was conducted on December 20 2021-January 20 2022. This research was carried out at the Brackish Water Cultivation Installation (IBAP) of Lamongan Regency. Measurement of water quality and observation of embryogenesis and egg hatchability of the Srikandi tilapia strain were carried out at the Brackish Water Cultivation Installation Laboratory (IBAP) of Lamongan Regency.

Tools and Materials

This research activity was supported by using several tools and materials used in conducting research at the Brackish Water Cultivation Installation (IBAP) of Lamongan Regency. Several types of equipment used in this study were aquariums with a size of 300x100 cm, aeration hoses, aerator stones, DO meters, pH pens, microscopes, petri dishes, pipettes, digital scales, stopwatches and incubators.

The materials used in this study included the mother and eggs of the Srikandi tilapia strain, NaOH, acetic acid (CH₃COOH), tissue and distilled water.

Experimental design

The experimental design in this study was a completely randomized design (CRD). The treatment used was the pH of the maintenance medium with four repetitions each. Measurement of water quality was carried out for five days during the research activities. The experimental procedure written by the author must be presented clearly.

Research procedure

Broodstock Selection

The criteria for a good male parent (*Oreochromis aureus*) are having undamaged scales, not physically weak, not physically disabled, not infected with disease, mature gonads which can be identified by the reddish protruding genitals. The male parent has a larger body shape compared to the female parent. While the female parent has characteristics on her genitals that are red and do not stand out. Mature female gonads are marked with transverse lines on the genitals and are red in color.

Spawning

The spawning process was carried out with a ratio of 1:3, namely 100 male (*Oreochromis aureus*) and 300 female (*Oreochromis niloticus*). Broodstock reared in a pond with a size of 250 m². Tilapia has the property of incubating its eggs in the mouth (mouth breeder) which is carried out from the time the eggs are fertilized until they hatch, which is for 2-3 days. Spawning time is about 18 days. In this study, fertilized tilapia eggs were transferred to an incubator that had been prepared previously. The research method was written briefly and clearly with the time of research and the methods used in the study.

Treatment of Different pH in Hatching Media of Srikandi Tilapia Fish Eggs

This study used 4 treatments, treatment (pH 6, pH 7, pH 8 and pH 9) repeated 4 times. Preparation of the hatchery media begins with preparing 4 aquariums, 16 incubators and installing aeration hoses and aerators. The initial pH used in this study was 7, the material used to increase the pH value is NaOH and the material used to lower the pH value is acetic acid (CH₃COOH). After the egg is fertilized by sperm, the egg is placed into an incubator of 10 eggs for each sample. Maintenance media can be seen in Figure 1.



Figure 1. Maintenance media

Embryogenesis observations were made at the 4th, 45th, 76th, 86th and 100th hours after fertilization occurred. Calculation of Hatching Rate using the following formula:

$$HR = \frac{\sum \text{hatched eggs}}{\sum \text{fertilized eggs}} \times 100$$

Survival rate is the percentage ratio between the number of living organisms at the end of the period. Survival rate calculations were carried out from hatching to 2 weeks age of larvae. Survival rate can be calculated using the following formula (Jaya et al., 2013):

$$SR = \frac{N_t}{N_o} \times 100\%$$

Information:

SR = degree of survival (%)

N_t = number of fish larvae at the end of rearing (heads)

N_o = number of fish larvae at the beginning of rearing (tails)

Data analysis

Observations of embryogenesis and hatchability of fish eggs were statistically analyzed using ANOVA and continued with Duncan's Multiple Range Test if the results of the ANOVA test resulted in a significant effect of the treatment given (Siregar et al., 2018). Water quality measurements were carried out during the research activities. Parameters observed were temperature and dissolved oxygen.

3. Results and Discussion

Srikandi Strain Tilapia Embryogenesis

Fish eggs will undergo an embryogenesis process, namely the process of egg development starting from the cleavage, morula, blastula, gastrula and organogenesis phases to the hatching process (Mubarokah et al., 2014). Embryogenesis of tilapia four hours after fertilization of all treatments was at the end of the cleavage or morula period. In the morula phase, the blastomers that are formed will condense until they become blastodisks at the poles of the anima and then form two layers of cells (Redha et al., 2017). Embryogenesis of Srikandi tilapia eggs four hours after fertilization can be seen in Figure 2.

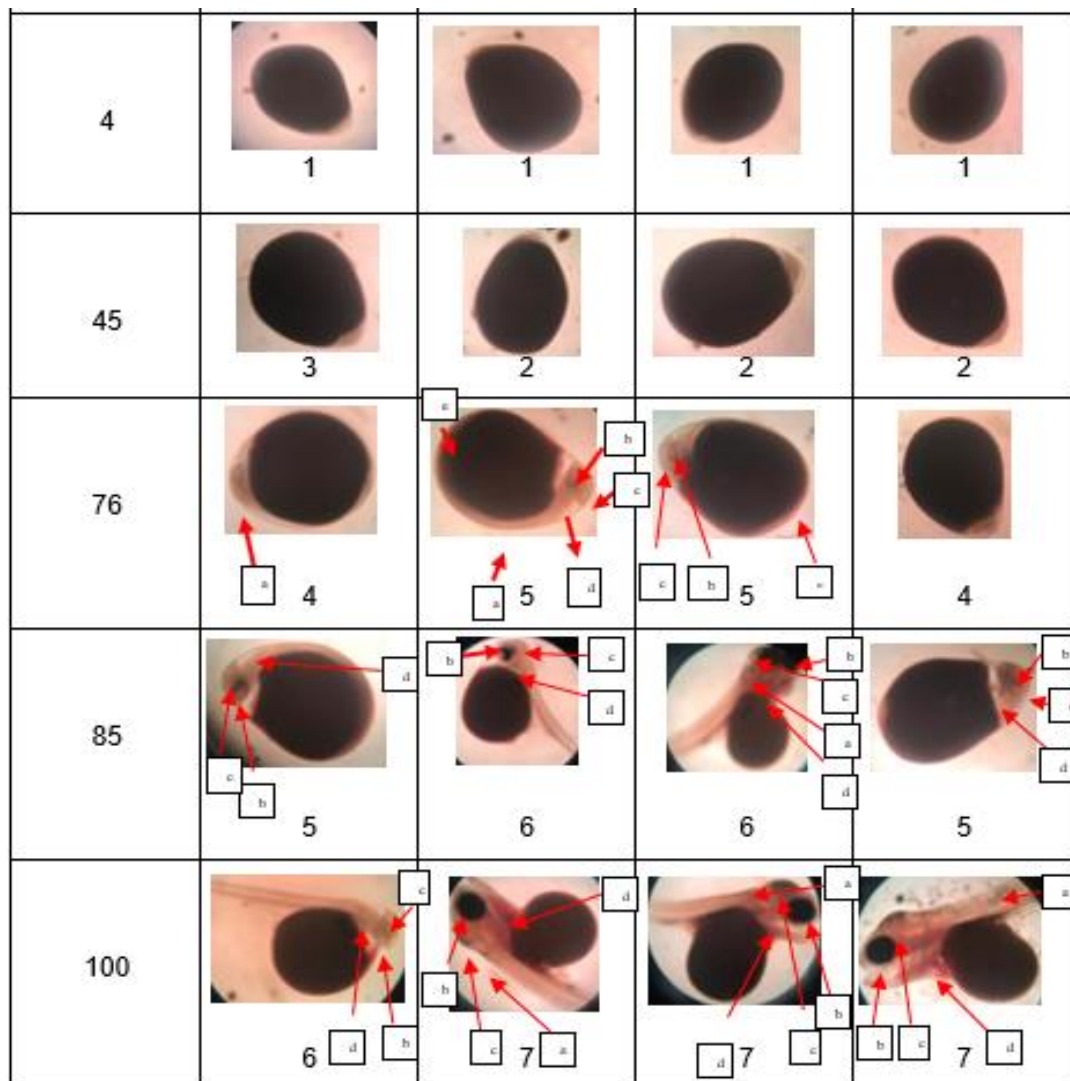


Figure 2. Embryogenesis of srikandi tilapia eggs at different pH with 40 times magnification.

(Note : 1. The end of the cleavage period; 2. The beginning of the blastula period; 3. The end of the blastula period; 4. The eyes are visible but not yet pigmented and there are already melanophore

spots on the surface of the egg; 5. The eyes are already pigmented, the heart looks throbbing, the brain begins enlarged, the tail appears elongated ventrally on the germinal ring; 6. Larvae 0 days old; 7. Larvae 1 day old; a. melanophore spots; b. eyes; c. brain; d. heart; e. germinal rings).

Embryo development at 45 hours after fertilization showed that treatment A (pH 6) had entered the late blastula phase, while the tilapia embryos in treatments B (pH 7), C (pH 8) and D (pH 9) had just entered the early blastula stage. In treatment A, the embryogenesis process was accelerated due to the different conditions of the hatching media which resulted in the inability to tolerate the existing conditions. Embryogenesis of Srikandi tilapia eggs at 45 hours after fertilization can be seen in Figure 2.

Tilapia embryogenesis at 76 hours after fertilization occurred, tilapia embryos in treatments B and C showed pigmented eyes, the heart was seen beating, the brain began to enlarge, the tail appeared to elongate ventrally in the germinal ring, whereas in treatments A and D the embryos showed part of the eye that is visible but not yet pigmented and has melanophore spots on the surface of the egg. Embryogenesis of Srikandi tilapia eggs at 76 hours after fertilization can be seen in Figure 2.

The development of the Srikandi tilapia embryo 85 hours after the fertilization stage, the tilapia embryos in treatments B and C were seen to have hatched and the 0 day old larvae still had yolk sacs, whereas in treatments A and D they only showed pigmented eyes, the heart seemed to beat, the brain begins to enlarge, the tail appears to elongate ventrally at the germinal ring. Embryogenesis of Srikandi tilapia eggs at 85 hours after fertilization can be seen in Figure 2.

The development of the Srikandi tilapia egg embryo one hundred hours after fertilization in treatment A (pH 6) showed that the eggs had hatched and the larvae were 0 days old and still had a yolk sac, whereas in treatments B (pH 7), C (pH 8) and D (pH 9) the larvae began to develop and were one day old and still had a yolk sac as in treatment A. Embryogenesis of Srikandi tilapia eggs one hundred hours after fertilization can be seen in Figure 2.

Lowering the pH using acetic acid which, when mixed with water, causes the chorion to become corrosive and causes the eggs to not develop. The use of NaOH to increase the pH value in large quantities results in delays in the embryogenesis process because the chorion has pores and the NaOH present in the incubation medium can enter the egg and inhibit the embryogenesis process. The chorion layer contains lime if the hatching medium contains a lot of NaOH it will cause the shell to become hard and embryonic development to be limited resulting in the death of the egg. The pH value affects the process of hatching fish eggs (Putra et al., 2020). High levels of acid compounds or high levels of bases present in waters can disrupt the process of fish development (Saleh et al., 2013).

Hatching rate of Srikandi Tilapia Eggs

Calculation of hatchability of tilapia eggs one hundred hours after fertilization. Data on the average hatchability of tilapia eggs at the end of the study are in Table 1.

Table 1. Hatching Rate of tilapia eggs at different pH

Treatment	Hatching Rate (%)±SD
A (pH of 6)	55±5,774 ^a
B (pH of 7)	87,5±5,000 ^b
C (pH of 8)	80±8,165 ^b
D (pH of 9)	50±8,165 ^a

note: Different superscripts in the same column and row show a significantly different effect ($P < 0.05$).

The highest average hatchability of tilapia eggs was produced by treatment B, which was 87.8%, followed by treatment C at 80%. Furthermore, treatment A was 55% and treatment D produced the lowest average hatchability of fish eggs by 50%

The results of the Analysis of Variance (ANOVA) showed that the hatchability of tilapia eggs hatched at different pH treatments gave very significantly different results ($P < 0.05$). Duncan's Multiple Range Test results stated that treatments A (pH 6) and D (pH 9) were very significantly different from treatments B (pH 7) and C (pH 8). However, treatment A (pH 6) and treatment D (pH 9) were not significantly different, and treatment B (pH 7) and treatment C (pH 8) were not significantly different either. The highest average hatching rate of Srikandi tilapia eggs was in treatment B (pH 7).

The factors that affect the hatchability of fish eggs are internal factors consisting of hormones and yolk volume and external factors consisting of water quality parameters such as temperature, pH, salinity, dissolved oxygen and light intensity (Gusrina, 2008). Internal factors in this study were the use of eggs from the same parent and in the same amount so as not to affect the embryogenesis and hatchability of tilapia eggs, while the external factors in this study were the use of the same aquarium and environmental conditions, which were different. only at a given pH condition.

A pH that is too acidic results in a tug-of-war between the nutrients from inside the egg and its environment, this makes the eggs hatch faster or even cannot hatch. Eggs that have been fertilized by sperm will be clear or transparent in color (Putri et al., 2013). Water conditions that tend to be acidic result in delays in the process of developing eggs until they hatch (Wardani, 2017). A pH that is too high causes the outer shell to have a high lime content and causes the eggs to hatch longer because the nutrients from outside that the eggs need cannot enter and conversely the nutrients that are not needed by the eggs cannot get out. The majority of aquatic organisms will be sensitive when the pH fluctuates and aquatic organisms prefer a neutral pH between 7-8.5 (Nasution et al., 2021).

Wardani (2017) conducted at different pH had a very significant different effect on the hatching speed of Baung fish (*Mystus nemurus*), egg hatchability and larval survival. As well as significantly different effect on abnormalities. The fastest egg hatching time was obtained at pH 8 with a hatching time of 29 hours 47 minutes. The highest percentage of hatchability was obtained at pH 9 of 82.22% and the lowest larval abnormality value was obtained at pH 9 of 4.48%, while the highest percentage of survival was at pH 9 of 79.36%.

A similar study was also conducted by Widura (2019), different pHs had a very significant different effect on the hatching speed of wader ray fish (*Rasbora argyrotaenia*) eggs. The fastest development of wader ray embryos occurred in the pH 8 treatment but the hatching time was the slowest around 30 hours 16 minutes. pH 8 gives a 76% effect on the degree of fertilization

Water temperature

Based on the average temperature data in Figure 3 which was measured during the study, the highest temperature was in treatment A (pH 6) of 28.46°C, while the lowest average temperature was in treatment D (pH 9) of 27.7°C. This value is still in the good category if used for fish maintenance (Setiawan et al., 2013). Sudden changes in temperature and high temperatures can cause delays in the hatching process and can result in death. Temperatures that are below 20 °C or above 30 °C can cause fish to experience stress and decrease the digestibility of fish (Nugraha, 2012). The higher the temperature, the faster the embryo development process (Mustaqim et al., 2019).

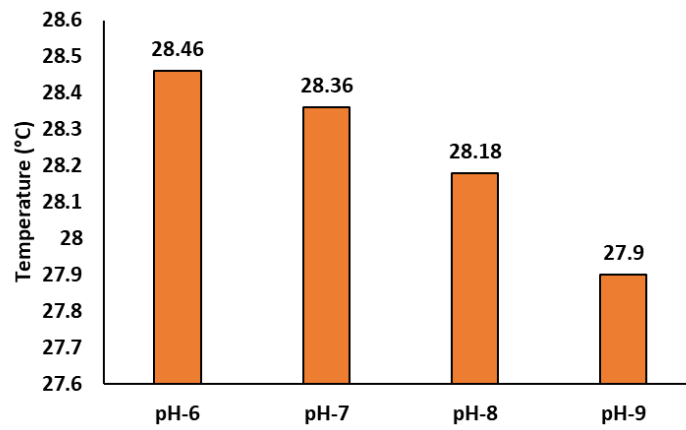


Figure 3. Average temperature in all treatments

Dissolved oxygen

Based on the average dissolved oxygen data in Figure 4 which has been measured during the study. The highest average dissolved oxygen was in treatment A (pH 6) of 4.76 mg/L, while the lowest average dissolved oxygen was in treatment C (pH 8) of 4.36 mg/L. A good dissolved oxygen value for hatching fish eggs is not less than 4-5 mg/L (Aryani, 2015). Low dissolved oxygen content can inhibit embryo development and can even cause death in the embryo.

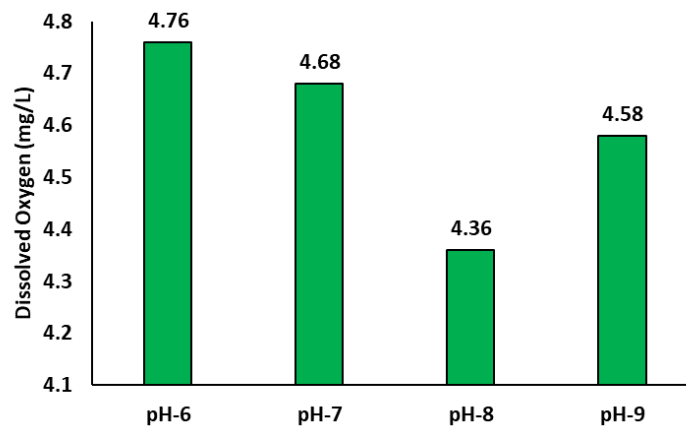


Figure 4. Average dissolved oxygen in all treatments

Survival rate

Survival rate or survival rate is the percentage of fish that live from the number of fish reared during research activities. Survival rate calculations were carried out from hatching to 2 weeks age of larvae. The average survival rate data for all treatments can be seen in Figure 5.

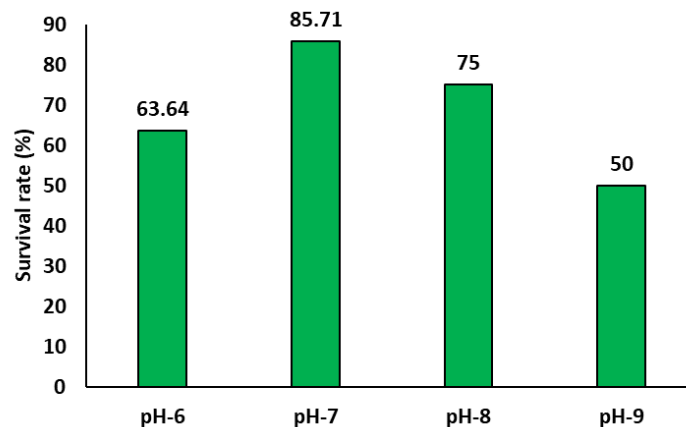


Figure 5. Average survival rate for all treatments

Based on the picture above, the survival rate for treatment A (pH 6) was 63.64%, treatment B (pH 7) was 85.71%, treatment B (pH 8) was 75% and treatment D (pH 9) was 50%. Survival rate can be influenced by external factors and internal factors (Pratama and Susilowati, 2018). A pH that is too acidic causes the survival rate to be low. The survival rate value can be affected by changes in the pH value in the rearing medium (Nisa et al., 2013).

4. Conclusion

Based on the results and discussion above, it can be concluded that pH has an influence on embryo development, hatching rate of the Srikandi tilapia strain. Meanwhile, the best pH to produce the highest hatchability of the Srikandi tilapia strain was in treatment B (pH 7) of 87.5%.

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