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**2020**

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**2nd International Conference on Fisheries and Marine Science 26 September 2019, Surabaya, Indonesia**

Accepted papers received: 16 January 2020

Published online: 24 February 2020

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# Effect of eggs immersion in tanin solution against embryonic development of common carp fish (*Cyprinus carpio* L.)



IOP Conf. Series: Earth and Environmental Science **441** (2020) 012155 doi:10.1088/1755-1315/441/1/012155

# Effect of eggs immersion in tanin solution against embryonic development of common carp fish (*Cyprinus carpio* L.)

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**Abstract.** Common carp (Cyprinus carpio L.) is a freshwater fish that has great potential to be developed, because it have high nutritional content. The eggs carp that is cultivated naturally have a high mortality rate, almost 80. Tannins have the ability to bind and precipitate proteins the caused by the presence of a number of functional groups that will bond strongly with protein molecules interacted which in turn will generate substantial crosslinking and tannin-protein complex that.This study was conducted to determine the effect of soaking the eggs in a solution of tannin on the embryonic development of carp. Method used in this study using quantitative descriptive method. The results showed that the soaking time tannin solution can affect to the rate of embryonic development of common carp at each phase. In the treatment of 120 and 180 seconds embryos develop faster in the early phase and the final phase is slower than the control. This suggests that the tannin solution can Accelerate the development of the embryo in the early phase and the discovery of abnormal larvae hatch in the treatment of 180 seconds.

## 1. Introduction

Carp (*Cyprinus carpio* L.) is a freshwater fish that has great potential to be developed, because the carp have a fairly high nutrient content. Punten variety carp which has the advantage of fast growth by having good and thick meat quality but, in increasing the production of carp there are inhibiting factors [1]. Goldfish eggs are adhesive which is easy to stick eggs after the hardening process of the shell so that the egg will easily stick to the substrate such as leaves, plant roots, and others. According to Slembrouk et al [2], adhesive type of fish eggs is most likely a factor in egg quality, which causes a low degree of egg hatching. The nature of eggs like this, it is necessary to make an effort to overcome this problem by providing a solution to remove the egg adhesion.

The mucous layer of goldfish eggs is sticking which causes the fish eggs to clot so that the pores are closed causing death. The death of goldfish eggs can be suppressed by artificial fertilization through removal of the mucous layer of the egg after fertilization by washing with tannin solution or pure papain solution [3]. Tannins have the ability to bind and precipitate protein compounds caused by the presence of a number of functional bonding groups that will interact strongly with protein molecules which in turn will produce large and complex cross-bonds namely tannins-proteins. Tannins can cause eggshells to become harder and embryo hatching more difficult [4]. Based on the description above, research on the

effect of soaking eggs in tannin solutions on the development of goldfish embryos needs to be done to determine the effect of tannins on goldfish embryos.

## 2. Material and methods

### *2.1 Fertilization of goldfish*

Immersion treatment on tannin solution is done after fertilization process, because if soaking is done before fertilization will cause the perivitellin layer on the egg to expand and can result in a micropyle layer on the egg to be closed so that the chance of sperm to be able to fertilize the egg is getting smaller. The process of immersion in the tannin solution begins with the spreading of the eggs in a sieve in the tannin solution then rinsed with fresh water. Goldfish eggs that have been soaked in a solution of tannin and rinsed with fresh water and then filter that contains eggs and rinsed transferred in the incubation container.

### *2.2 Incubation and observation of the development of goldfish embyos*

Goldfish eggs can hatch at temperatures of 24-30$^{o}$ C with longer incubation 72-120 hours. Embryogenesis is the process of formation of living organisms that do not have a form that characterize a living thing. Embryogenesis process starts from the stage of cleavage (cleavage), blastula, gastrula, segmentation and organogenesis, and hatching. These observations were made to determine differences in embryo development in each treatment. Observation of early embryos is done every 5 minutes and then at the end of the embryo phase carried out every 2 hours. These observations were made by using a binocular microscope. Changes that occur also recorded and documented using a digital camera.

## 3. Result and discussion

### *3.1 Egg characteristics goldfish (Cyprinus carpio L.)*

The characteristics of the post-immersion goldfish eggs is by observing the size of goldfish eggs and egg on mikropskop sightings. Diameter of the immersion of 0 seconds which is 1.3 mm, whereas the soaking 120 seconds has a diameter of 1.5 mm and at 180 seconds immersion has a diameter of 1.5 mm. Visible differences between the non-treated by soaking treatment of 120 seconds and 180 seconds. On a given treatment eggs undergo development or enlargement of the yolks. The image of eggs that had been given treatment which can be seen in Figure 1.

(a) (b) (c)

**Figure 1**. Egg goldfish with treatment (a) 0 seconds; (B) 120 seconds; (C) 180 seconds

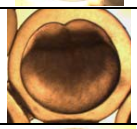
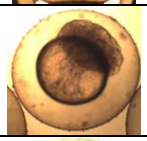
The size of carp diameter is in accordance with the statement of [5], the diameter of carp eggs is around 1.14 mm to 1.42 mm. However, there are differences in observations on the shape of goldfish eggs that have been fertilized. In goldfish eggs soaked in tannin solution experiences enlargement in the yolk and protein deposition, because tannin and protein have functional bonds. According to [6], egg yolk sacs

are membranes that provide nutrients for embryos. The occurrence of enlargement of the yolk is one of the factors in the absence of a good distribution of nutrients for the embryo. This will gradually cause the embryo to die.

*3.2 Egg development goldfish (Cyprinus carpio L.)*

The results of observations that have been made to determine differences in the development of goldfish eggs between eggs without treatment with eggs carried out soaking in tannin solution for 120 seconds and 180 seconds. Observation of egg development starts from zygotes to newly hatched larvae. The development of goldfish embryos includes eggs after fertilization (zygote), cleavage, blastula, gastrula, segmentation and hatching period. The fertilized egg forms a perivitellin space that separates the egg from the egg membrane. The difference in the time of development of goldfish embryos can be seen in Table 1. The development stage of goldfish embryos starts from zygotes or one cell that is marked by the formation of a single cell (blastodisc) on one side (animal pole) of the egg looks denser than the egg yolk (vegetal pole). Cell division begins with the division of single cell mitosis and produces two cells smaller than a single and the same cell. The stage of development of the blastula is characterized by an invasion of the yolk, resulting in a germinal ring and a portion of the yolk has not been covered by the blastomer. The next stage is the stage of segmentation and organogenesis. This stage begins with the formation of eyes and tails. Subsequent developments occur in the formation of the head, tail, spine, vertebrae, ovaries, heart and eye pigmentation. The final phase is the hatching phase marked by the hatching of carp larvae.

**Table 1.** Time Egg Goldfish Embryonic Development (*Cyprinus carpio* L.) with a 100x magnification microscope

| Phase | | Embryonic Development Time | | |
|---|---|---|---|---|
| | | Control | 120 Seconds | 180 Seconds |
| **zygote** |  | (06:00) | (06:00) | (06:00) |
| **Cleavage (Clevage)**<br>2 cells |  | (06:20) | (06:10) | (06:10) |
| **blastula**<br>128 cells |  | (08:55) | (08:05) | (08:25) |
| **gastrula**<br>50% Epiboly |  | (12:55) | (14:00) | (15:15) |
| **Segmentation and Organogenesis**<br>3-6 somite |  | (16:55) | (00:00) | (01:15) |
| **Hatch** |  | (02:00) | (02:10) | (02:20) |

Observation of the development of goldfish embryos consists of 6 phases, namely egg after fertilization (zygote), cleavage, blastula, gastrula, segmentation and organogenesis and hatching period. Like the statement Bie et al. [6] which states that the development of koi fish embryos are divided into six periods, namely zygote, cleavage, blastula, gastrula, segmentation and hatching period. Each period consists of several phases.

Eggs that have been fertilized with egg yolks begin to develop into one cell, also called zygote. This is consistent with the statement of [7] that newly fertilized eggs enter the zygote period until the first division occurs. In this period, the cytoplasm moves towards the animal pole to form the blastodisk.

The cleavage process is a process in which the yolk splits which starts from two cells to divide into 64 cells. This is consistent with what was stated by Kimmel et al. [7] which states that the division period begins when the blastodisk has formed and divides into two until the division of 64 cells. According to [8] Cleavage is a process of division of an embryo that has undergone mitotic fertilization. The process of division only divides the cytoplasm into many smaller cells with their respective nuclei, called blastomers. During cleavage, the total volume of embryonic cells is the same or no increase in size, only the number of cells increases.

The blastula phase is where the egg begins to divide into 128 cells, then becomes many cells and up to a 30% epiboly phase. The blastula process is a process in which cells that have been divided into one to form a cavity. This is consistent with the statement of Kimmel et al. [7] that the blastula period begins when the blastodisk begins to look like a ball in the 128 cell phase and ends in the 30% epiboly phase. The egg dividing into 128 cells occurs in 2 hours 30 minutes. Eggs develop into oblong phase takes 3 hours 5 minutes from the division phase of 128 cells. [9] stated that in the development of incubated koi fish embryos at temperatures 26º-28º C, the blastomer began to cover the yolk (yolk) at 3 hours after fertilization.

The gastrula phase is a phase that begins with a 50% epiboly phase and ends in the bud phase, where the bud phase is the phase where the eyes and tails will be seen. Kimmel et al. [7] explain that late gastrula appears when epiboly has covered 80-90% of egg yolk. According to Haniffa et al. [9], koi fish embryos incubated at 26º-28º C, the blastomer almost covered all parts of the yolk at 5 hours 26 minutes after fertilization. The head and tail can also be distinguished.

The segmentation and organogenesis phase is a process in which in this phase the spine axis (notochord) and vertebrae (somite) begin to form. According to Haniffa et al. [9], in koi fish embryos that were incubated at 26º-28ºC, the notochord was clearly seen with the number of somite of 22 segments occurred 14 hours after fertilization. [10] which states that the emergence of eye pigment is marked by the black color found in the eye. In the final phase of goldfish organogenesis shows that the eye pigmentation that starts perfectly starting from the black color is evenly distributed in the eye. Blood circulation goes faster and the embryo has filled the inside of the egg so that the tip of the tail appears to fold or overlap the head. The movement of the embryo is faster toward the side or spinning. This embryo movement indicates the embryo will hatch.

The last stage of development of the goldfish embryo is the hatching stage, where the embryo has fully developed and is ready to hatch. In the development of goldfish embryos with a period of 48 hours. This is in accordance with the statement of [11] states that koi fish embryos hatch into larvae at 36-48 hours after fertilization with temperatures of 28º-29ºC. The development of goldfish embryos during observation is slower due to differences in species observed and differences in temperature that affect embryo development, according to the opinion of Haniffa et al. [9] which states that the length of incubation period of fish eggs is not the same, depending on the fish species and some external factors, especially temperature the waters.

*3.3 Eggs and larvae goldfish abnormality*

In this research, the discovery of eggs and larvae of carp abnormal (defective) at 180 seconds soaking treatment. Abnormal larvae (defects) are likely to be due to a problem during cell division. Abnormal larvae can survive such movement can be seen swimming round and round like a loss of balance. Larvae abnormal (defective) is usually caused by the bending of the spine and tail. The image of eggs and larvae of carp abnormal (defective) which can be seen in Figure 2.

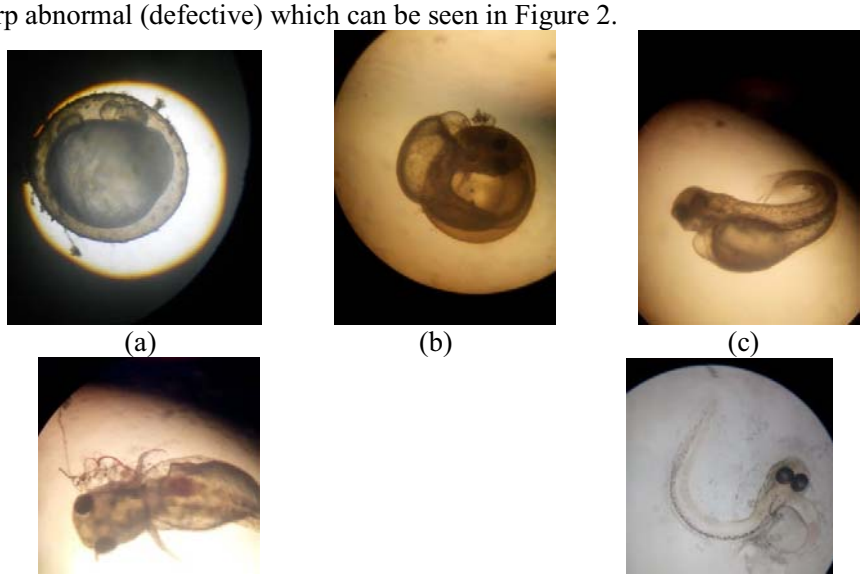



**Figure 2.** Eggs and larvae of carp abnormal (defective): (a) Form of abnormal eggs; (B) The larvae are difficult to hatch; (C) The bending of the bones on the larvae hatch body; (D) The tissue is damaged in the newly hatched larvae; (E) The yolk larvae were broken.

According to Pudjirahayu et al [12], explained the characteristics of abnormal larvae, namely the body shape of the bent larvae and abnormalities in the shape of the head, tail and short body size so that the larvae cannot move actively. According to Kim [13], the bending of the joints of the body can interfere with swimming and disrupt eating. According to Kujawa et al [14] suggested that using tannin for too long is dangerous for embryonic development, because the egg membrane inhibits the embryo's ability to hatch. Even though they are fully ready to hatch, they cannot break the egg membrane so that the embryo dies. The egg membrane is partially damaged and cannot free itself, the embryo will die. High tannin concentrations can cause the most severe problems in the hatching process, because they can cause high larval death.

*3.4 Water quality parameters*

Water quality is one of the parameters that can affect the process of hatching fish eggs. In this study, water quality was observed which includes temperature, dissolved oxygen (DO), and pH. Monitoring of water quality is done twice, at the beginning before the eggs in the incubation and at the time the eggs were incubated. Monitoring of water quality should be done twice a day ie morning and afternoon hari. Water quality measurement results during the research can be seen in Table 2.

Water quality is another factor that can affect the process of fish embryo development. The qualities that must be considered in fish culture and in the process of embryo development are temperature,

dissolved oxygen (DO), and pH. In research water quality is quite good water before the eggs are incubated and after the eggs are spread on the incubation media. Water quality at the time of observation is with a temperature of water ranging from 24º-27ºC, dissolved oxygen around 5-6 mg/l and an average pH of 8. In this study carp eggs hatched within 48 hours 15 minutes. This is consistent with the statement of [15] the rate of hatching of goldfish eggs occurred after 46-144 hours after fertilization at 25° C in the tropics. Meanwhile according to [16] states that koi fish embryos hatch into larvae at 36-48 hours after fertilization at temperatures of 28°-29° C.

**Table 2.** Results of water quality before and during the goldfish eggs incubated

| Temperature (0C) | Before the Egg Incubation | | During incubation eggs | |
|---|---|---|---|---|
| Days to - | 08:00 | 16:00 | 08:00 | 16:00 |
| 1 | 24.7 | 25.1 | 25.5 | 26.3 |
| 2 | - | - | 26.1 | 27 |
| 3 | - | - | 25.1 | 26.4 |
| Dissolved oxygen (mg / L) | 08:00 | 16:00 | 08:00 | 16:00 |
| 1 | 5.4 | 5.6 | 5.2 | 5.5 |
| 2 | - | - | 5.8 | 5.9 |
| 3 | - | - | 5.2 | 5.6 |
| pH | 08:00 | 16:00 | 08:00 | 16:00 |
| 1 | 8.3 | 8.2 | 8.3 | 8.3 |
| 2 | - | - | 8.3 | 8.2 |
| 3 | - | - | 8.2 | 8.2 |

## 4. Conclusion

The results of observations to determine the effect of soaking goldfish eggs in tannin solutions on the development of goldfish embryos can be seen that soaking eggs in tannin solutions affect the rate of change in the development phase of the embryo and found abnormal growth of eggs and larvae.In the study the effect of soaking time of goldfish eggs in tannin solutions on embryo development with 120 and 180 seconds treatment is not the optimum time.

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