Different of saline embriogenesis and eggs hatching rate of the giant gourami (Osphronemus gouramy)

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Abstract. Water salinity is one of the environmental factors that affect the hatching rate of the fish. The aims of this study were to find out the influence of salinity to embryogenesis and hatching rate of giant gourami at the different salinities. This study used four treatments and three replications. The treatments were A 0 ppt, B 1-2 ppt, C 3-4 ppt, and D 5-6 ppt. The salinity was made by adding salt water to the fresh water in aquarium. The number of eggs in each treatment was 32 grains respectively. The parameters observed were the embryogenesis, fertility, hatching rate, hatching time, and water quality. The results showed that salinity influenced the embryogenesis duration of eggs during blastula and gastrula stages, but did not influence to the hatching rate and hatching time of eggs.

1. Introduction

The giant gourami is one of the fresh water fisheries commodities which have high demand in fish market. The price of gourami was increasing from 2014 at Rp 28.627,- to Rp 31.709,- in the producer while in the retail market from Rp 36285 to Rp 40724 [21]. However, the production target of the giant gourami in 2011-2014 by the Ministry of Fisheries and Marine Affairs (MFMA) was not achieved. It might be caused by the centralized breeding activities and the low interest of the peoples to culture the giant gourami due to their low growth rates [5]. Therefore, the MFMA initiated the strategy of segmentation in fish culture in order to increase the development of fish culture area which automatically needs the seed of fish in good qualities, quantities as well as their continuities. The success of fish seedling is related tightly to the process of egg embryogenesis. Embryogenesis and its individual characteristics accordingly was affected by the genetic factors and environmental factors [14]. One of the environmental factors was the salinity [8]. The salinity will affect the osmoregulation of the fish eggs in their hatching processes, where the fresh water fish eggs which were kept in the intolerable salinities would wrinkled the eggs due to the water was pull out from the eggs and finely died [19]. However, it is revealed that in the isoosmotic salinity condition the energy used in osmoregulation was very minimum [2]. As a consequence, the portion of energy for activity and growth process increased and affects to the embryogenesis and eggs hatching rate. The previous research showed that there was a significant effects of the salinity treatment on the embryogenesis and hatching rate of several fishes [4, 10]. The information about the effect of salinities on the embryogenesis and eggs hatching rate of the gourami was not known yet. Therefore, it is necessary to study the effects of...
salinities of the incubation process on the embryogenesis and eggs hatching rate of the giant gourami to know the best salinity condition in producing the optimum egg hatching rate.

2. Material and Methods
Materials and apparatus used in this study were aquarium, refractometer, thermometer, water container for saline water, hatching funnel, drop pipettes, pH meter, petri dishes, microscope and camera. The materials used for this studies were kitchen salt, Sera test kit for O₂, and eggs of the giant gourami. The experimental design used in this research was Randomized Complete Designed with 4 treatments and 3 replications. Salinity treatment in each aquarium were A (0 ppt), B (1-2 ppt), C (3-4 ppt), dan D (5-6 ppt). The Eggs numbers in each aquarium was 32 eggs obtained from natural spawning of the brood stocks.

2.1. Experimental procedure
The brood stock of the giant gourami was obtained from the Mini Raiser of the Fresh water and Insect world, Beautiful Indonesia Miniature Park, Jakarta. One male and one female of brood stocks were used in this experiment, which have been selected and then reared in the earthen pond till they hatched naturally. They were fed with commercial pellets added with Vitamin E (1 gr/ 2 kg Feed) and in shift added with leaves of calladium (*Alocasia macrosrhitia*) to increase the eggs qualities. The feeding was given twice a day at 09.00 and 16.00 in ad satiation. The size of aquarium used was 36.4 x 24.2 x 35 cm. They were cleaned and sterilized with formaldehyde in 2 ppm for 24 hours and then rinsed properly. Furthermore, the hatching funnel was settled, along with thermometer, and container of the saline water. Salinity of the water medium was obtained by adding the salt solutions at 37 ppt to the aquarium which have been filled with fresh water in phases from the time of eggs added to the aquarium. Salinity of the water media on the treatments B, C, and D were increased as the increment of saline water to the aquarium during 3 hours. The use of salt waters due to the efficiency process in this study as well as for practical purposes in the fields. The scheme of the hatching apparatus for gourami eggs presented in Figure 1.

2.2. Embryogenesis
Observation was started from the time of transferring the eggs from the nest [23]. The observation of embryogenesis was done every hour in the first day, and every 3 hours in the second day. About 6 % of sample was taken from the population for observation, with the assumption that the fertile eggs were from the male and female brood stocks which has highly uniformity.

Fertilized eggs were counted one by one, and then set by using the following equation [8].
Calculation of the hatching rate was determined after all of the eggs fertilized was calculated by the following equation [8]

\[
Fertility\ rate = \frac{\text{Amount of fertilized eggs}}{\text{Amount of eggs}} \times 100\%
\]

[1]

Hatching time was determined by calculating the range of time when the eggs were put on the incubation funnel and the time when all of the eggs hatching [18]. Water quality parameters measured in this experiment were salinity, water temperature, pH and dissolved oxygen (DO). Measurement was conducted every three hours since the addition of saline water into the incubation medium where was carried out in simultaneously with water temperature measurement. pH and DO measurement was done once a day at 10 am.

3. Results and Discussions

3.1. Embryogenesis

The number of eggs obtained from natural spawning of a couple of giant gourami brood stock was 421 eggs where the 391 eggs (92%) were in viable condition and 30 eggs (7.2%) were died. The eggs were taken from the nest and then observed under the microscope. Results of the observation of embryogenesis of the giant gourami was presented in the Table 1. The blastula stage in treatment A was occurred in a shorter period compared to another treatments. The blastula stage of the rainbow fish (Melanotaenia parva) without salinity treatment takes place in shorter period compared to that of the saline treatment in incubation medium [15]. The embryogenesis of several strains of gourami have been studied by early workers showed the same results [20, 23]. The different on embryogenesis stages occurred due to different ability of the eggs to adapt with the salinity of incubation media. Salinity in the waters emerging the osmotic pressure which may different from the osmotic pressure in the body of the organism. In this condition the organism have to suit the osmoregulation of their body to balance the osmotic pressure within the body, so that the energy in the body was much more allocated for the body metabolism, while the mechanism of osmoregulation was the response for the environmental change [17]. Furthermore, the energy for growth and development automatically decreased therefore the embryogenesis stages were different. It is revealed that the disturbance of salinity change may cause the different in embryogenesis duration and decreasing the hatching rate [3].

Table 1. Embryogenesis of the giant gourami eggs with three salinity treatments A (0 ppt), B (1-2 ppt), C (3-4 ppt), and D (5-6 ppt)

| Stage of Embryogenesis | Duration (from hour of - to the hour of-) |
|------------------------|------------------------------------------|
|                        | A  | B  | C  | D  |
| Blastula               |    |    |    |    |
| Cleavage               |    |    |    |    |
| Gastrula               |    |    |    |    |
| Tubular                |    |    |    |    |
| Stiphonema             |    |    |    |    |
| Hatching              |    |    |    |    |
Cleavage

3 hours | 7 hours | 8 hours | 8 hours
(3-5)    | (3-9)   | (3-10)  | (3-10)

Blastula

9 hours | 5 hours | 4 hours | 4 hours
(6-14)   | (10-14) | (11-14) | (11-14)

Gastrula

6 hours (15-20) | 6 hours (15-20) | 6 hours (15-20) | 6 hours (15-20)

Neurula

16 hours | 16 hours | 16 hours | 16 hours
(21-36)   | (21-36)  | (21-36)  | (21-36)

Organogenesis

7,3 hours | 9 hours | 9 hours | 8,3 hours
(36-44)   | (36-45) | (36-45) | (36-24)

Hatching

Notes: a: cell in cleavage stage, b: yolk sac, c: vegetative pole, d: previtelline space, e: blastomere in animal pole, endoderm, g: mesoderm, h: ectoderm, i: neural plate, j: tail bud, k: embryo body, l: tail.
3.2. Hatching rate
The hatching rate of the giant gourami eggs was presented in Figure 2. The different salinity treatment respectively from 0, 2, 4, 6, 8, and 10 ppt showed not significantly different on the hatching rate of the rainbow fish eggs [15]. The different of survival rate of the fresh water fish in different saline waters was depend on the surface width of the gill (Chloride cells), the oxygen consumption, tissues tolerance, and the ability to manage the ion concentration and osmotic pressure [2]. The regulation of the eggs osmotic pressure was done by the active transport through the chloride cells at the surface of the membrane of eggs plasma. In early stage of eggs and early larval stage the secretory organs was not developed yet. Therefore, the regulation was done by the ectoderm and a number of enzymes that associated with the membrane as well as in the cytoplasm [2].

![Hatching rate of the giant gourami eggs in different salinities](image)

**Figure 2.** Hatching rate of the giant gourami eggs in different salinities

The Salinity was known as supporting role in increasing the hatching rate of the giant gourami eggs. It is revealed that the salinity has a supporting role in decreasing the energy for osmoregulation for the eggs of the freshwater catfish compared to that of the temperature [11]. It was known that the salinity was not the main factor that affects the hatching process [26, 27]. The relationship of the egg osmoregulation and hatching was an analog with the osmoregulation in fish. The body liquid of the fresh water fish has a higher pressure that its environment therefore the salts tends to flow out of the body and on the contrary the water flow in to the body. For the normal physiological processes in the body osmoregulation activity must be carried out to obtain the constant osmotic pressure [17]. The salinity treatment was able to change the liquid concentration inside as well as outside of the egg to become lower or higher concentration, depend on the liquid condition inside the egg and its adaptation ability to the environmental condition.

3.3. Egg hatching time
Hatching time of the giant gourami eggs of this experiment is presented in the Figure 3. The same tendency was found in the Siamese catfish [11]. However, the salinity treatment has affected significantly for the hatching time of the marble goby [16] and the freshwater red tail catfish [10].
The change of environmental condition cause bring about the change in energy allocation in the fish body. The energy will be used for metabolic activities as the consequence of the change of environmental condition [1]. Several research results showed that salinity range for fish physiological processes, however it was different for each fish species in each habitat [7]. The optimal temperature and pH for giant gourami were 27-30 °C and pH about 7-8 [24] with DO around 5 mg/L [6].

4. Conclusion
The different salinity treatment affect the duration of the embryogenesis stage of the giant gourami eggs particularly in the blastula and gastrula stages. The salinity treatment did not affects significantly in increasing the hatching rate and hatching time of the eggs of the giant gourami.

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