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The different effects of heat shock duration and embryo age on embryonic development and hatching lengths of spotted barb (*Puntius binotatus*) fish

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Abstract. The study aimed to determine the different effects of heat shock duration and embryo age on embryonic development and hatching lengths of spotted barb fish. The method was used the experimental method using a factorial completely randomized design with two factors of treatment, namely heat shock duration (1, 1.5, and 2 min) and embryo age (3, 4, and 5 min after fertilization). The fish embryos were shocked at temperature of 40°C. Each combination was repeated three times. One treatment was conducted without heat shock as control. The results showed that treatments of heat shock duration and embryo age have affected embryonic development and hatching lengths of spotted barb embryo.

1. Introduction

Temperature is one of several determinant environmental variables that affect the development of fish embryos. Temperature causes embryonic mortality at extreme level and affects metabolic speed, including the maintenance and growth of fish embryos and larvae [1]. Temperature is one of the critical factors and controls during the development of the embryonic and larval phases in fish [2]. Several studies have been conducted related to the effect of temperature on hatching [3, 4]. The water temperature of the media during fish embryogenesis can affect the time and age of the embryo when it hatches [5].

Many studies about the effect of incubation temperature on embryonic development in fish have been conducted. However, the influence of temperature shock, especially heat shock in the polyploidization process on fish embryonic development has not been much studied. Until now, only few studies on embryonic death due to polyploidization have been reported include successful ploidy, such as [6, 7, 8, 9, 10, 11]. Although, the effect of cold shock triploidy induction on cell cleavage of spotted sand bass embryo has been carried out [8], however, the influence of heat shock on embryonic development has not been reported. Therefore, this study aimed to determine the effect of heat shock duration and embryo age on embryonic development and hatching lengths of spotted barb (*Puntius binotatus*) embryo.



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2. Materials and methods

This study was conducted to Technical Unit of Freshwater Aquaculture Development, Umbulan, Pasuruan, East Java, Indonesia and Wet Laboratory, Faculty of Fisheries and Marine, Universitas Airlangga, Surabaya, East Java, Indonesia.

2.1. Artificial fertilization

Eggs and sperm of spotted barb were collected at a clean and dry container separately, especially sperm was added physiological NaCl of 0.9% with ratio 1:9. Then, eggs were added sperm solution of 2-3 drops, stirred gently for 1 min, and added 1-2 mL freshwater at temperature of 27°C and stirred slowly for 1 min. On the other hand, fertilization rate was measured to determine egg quality of spotted barb fish before the treatment of heat shock.

2.2. Heat shock treatment

A number of 50 eggs were distributed in different filter according to each treatment and the number of 50 eggs were spread in control filter. The embryo age of 3, 4, and 5 min after fertilization (maf) were shocked at a water bath of 40°C temperature [13] for 1.0, 1.5, and 2.0 min, respectively.

2.3. Artificial incubation of embryo

The spotted barb embryos were incubated artificially at aquaria of 60×40×40 cm size. The water temperature was set around 27-28°C using thermostat. When 30-60 min after fertilization (maf), fish embryos were observed to initial embryonic development. Then, embryo sample was collected to observe the embryonic stage. The first hatched embryos were also observed.

2.4. Data analysis

Data were analyzed statistically and descriptively. Statistically, analysis was used analysis of variance (ANOVA). The treatment differences were determined using Duncan's multiple range test. Statistics are analyzed using SPSS 10 (statistical software). The significant level was determined at $p < 0.05$.

3. Results and discussion

The results showed that the fish eggs used were of good quality with an egg diameter of 0.1 to 0.25 mm and a fertilization rate of 76.92 to 89.38% and there were no significant differences between treatments and control ($p > 0.05$). This proves that the eggs used in this study are relatively homogeneous in terms of quality, so synchronization of egg fertility can be controlled properly. Fertilization observations were made at 4 h after the eggs were fertilized by sperm from the parent spotted barb fish. Determination of the success rate of egg fertilization is seen in the color change, ie the fertilized egg is transparent in color while the unfertilized egg is turbid white [14]. Genetic factors, feed, stress, and poor water quality are the causes of damage to egg quality [15] and can have an impact on survival during the early phases of life of several fish species [1].

Heat shock results in temperature fluctuations from low temperature to high temperature. Temperatures that are too high or too low can inhibit the hatching process, even those that are too extreme can cause embryonic death and hatching failure [16]. The results of microscopic observations note that the development of spotted barb fish consists of several stages, namely: cleavage, morula, blastula, gastrula, organogenesis, and hatching stages. The division stage starts from the first mitosis division to the fifth division. Nelsen [17] states that division is a process of development in embryonic cells which are cells increasingly becoming smaller or smaller units called blastomers.

The results showed significant differences ($p < 0.05$) between treatments of heat shock duration and embryo age, and their interaction on embryonic development and hatching lengths of spotted barb embryo (Table 1).

Table 1. The embryonic development and hatching lengths of spotted barb embryo after treated heat shock.

HSD+EA (min+maf)	Cleavage (h)	Morula (h)	Blastula (h)	Gastrula (h)	Organogenesis (h)	Hatching (h)
1.0 – 3	0.88±0.05 ^{cde}	2.56±0.08 ^{cd}	5.82±0.06 ^{ab}	6.97±0.24 ^a	18.10±0.46 ^a	21.63±0.25 ^a
1.0 – 4	0.87±0.04 ^{cde}	2.53±0.07 ^{bcd}	6.85±0.11 ^c	7.25±0.13 ^b	19.48±0.17 ^{bc}	22.00±0.97 ^{ab}
1.0 – 5	1.00±0.07 ^f	2.65±0.10 ^d	6.00±0.26 ^b	7.17±0.09 ^{ab}	19.30±0.28 ^b	21.81±0.81 ^{ab}
1.5 – 3	0.75±0.04 ^b	2.40±0.11 ^{ab}	5.85±0.87 ^{ab}	6.95±0.11 ^a	20.63±0.65 ^{de}	22.92±1.03 ^{ab}
1.5 – 4	0.81±0.08 ^{bcd}	2.48±0.72 ^{abc}	5.75±0.24 ^{ab}	7.00±0.13 ^a	18.50±0.43 ^a	22.00±1.00 ^{ab}
1.5 – 5	0.90±0.05 ^{de}	2.56±0.06 ^{cd}	5.92±0.15 ^{ab}	7.13±0.12 ^{ab}	18.20±0.16 ^a	20.73±1.18 ^a
2.0 – 3	0.66±0.61 ^a	2.38±0.04 ^a	5.58±0.33 ^a	6.97±0.08 ^a	20.83±0.14 ^c	22.59±0.89 ^{ab}
2.0 – 4	0.80±0.02 ^{bc}	2.50±0.09 ^{abc}	5.83±0.16 ^{ab}	7.00±0.05 ^a	20.00±0.70 ^{cd}	22.50±0.90 ^{ab}
2.0 – 5	0.83±0.04 ^{bcd}	2.46±0.05 ^{abc}	5.80±0.14 ^{ab}	6.97±0.11 ^a	19.28±0.12 ^b	21.77±0.84 ^{ab}
Control	0.92±0.03 ^{cf}	2.58±0.04 ^{cd}	5.83±0.09 ^{ab}	7.08±0.03 ^{ab}	19.25±0.15 ^b	22.41±0.28 ^b

Note: HSD = heat shock duration, EA = embryo age, maf = min after fertilization, h = hours. Different superscripts in the same column show no significant differences ($p < 0.05$).

The treatment of heat shock duration and embryo age have a significant effect ($p < 0.05$) on the initial division of two cells (cleavage). The treatment of heat shock duration of 2.0 min and embryo age of 3 maf being the fastest treatment in reaching the division stage of two cells (cleavage), while the treatment of heat shock duration of 1.0 min and embryo age of 5 maf became the longest treatment in reaching stage of two cells (cleavage). Iswahyudi [18] reports that the first hour after fertilization in spotted barb fish, while the first mitotic division in common carp (*Cyprinus carpio*) occurred within 20 maf [19].

The results of the ANOVA test for the treatment of heat shock duration and embryo age showed no significant difference ($p > 0.05$), with the time of hatching embryo of 20.73-22.92 h after fertilization. Iswahyudi [18] states that the hatching stage of spotted barb was achieved 24 h after fertilization. Meanwhile according to Haniffa *et al.* [19] showed that eggs of common carp hatch in 71 h 20 min after fertilization at 28°C temperature of incubation. The survival of the 4-day-old caked spotted barb fish larvae resulted from lower temperature shock treatment compared to controls. This is because the haploid embryo will die during hatching and only a small portion can survive [20]. Rustidja [21] states that the death of larvae is caused not only by the presence of defective larvae, but can also be caused by other factors such as water quality media, fungal attack, and inappropriate feeding. The difference in survival between controls and treatments may be due to differences in density in each treatment. Arai [22] states that differences in survival in fish given heat shock treatment, descriptively is not due to heat shock treatment given but rather is determined by the maintenance process carried out for example differences in larval density in each treatment, the nature of cannibalism or larval abnormalities.

4. Conclusion

Treatment of heat shock duration and embryo age influenced embryonic development and hatching lengths of spotted barb fish.

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