The different effects of heat shock duration and initial period on hatching rate, abnormality rate, egg yolk absorption, and survival rate of spotted barb (*Puntius binotatus*) larvae

A T Mukti¹,², M Ahmadi³, Widjiati¹ and E M Luqman³

¹ Department of Fish Health Management and Aquaculture, Faculty of Fisheries and Marine, Universitas Airlangga, Surabaya 60115, Indonesia
² Study Program of Aquaculture, Faculty of Fisheries and Marine, Universitas Airlangga, Surabaya 60115, Indonesia
³ Department of Veterinary Anatomy, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya 60115, Indonesia

Corresponding author: akhmad-t-m@fpk.unair.ac.id

Abstract. Spotted barb (*Puntius binotatus*) is one of Indonesian endemic fish with high economic value. However, this fish has slow growth, so innovative technology was required to increase fish growth. This study aimed to determine the different effects of heat shock duration and initial period on hatching rate, abnormality rate, length of egg yolk absorption, and survival rate of spotted barb larvae. This study was used an experimental method using factorial completely randomized design, with two factors of treatment, namely heat shock duration (1, 1.5, and 2 min) and initial period (3, 4, and 5 min after fertilization) of fish embryos. Egg fertilization was conducted artificially. The fish embryo was shocked at the temperature of 40°C. Parameters of hatching and abnormality rates, length of egg yolk absorption period, and survival rate were observed. Data were analyzed statistically using analysis of variance followed Duncan’s multiple range test with a confidence level of 95%. The results showed that the fertilization rate of the spotted barb was above 81%. Treatment of heat shock duration and initial period influenced hatching rate of fish embryo, and abnormality and survival rates of larvae; however, treatments have similar lengths of egg yolk absorption in spotted barb larvae.

1. Introduction

Spotted barb (*Puntius binotatus*) is one of Indonesian endemic fish with high economics value [1]. Spotted barb has several advantages as it is easily cultured and maintained in low dissolved oxygen content. However, slow-growing spotted barb has made culture development still under the other commodities. Increased production is required to fulfill the market demand adequately.

Chromosome set manipulation in fish is one of the expected strategies that can be used to produce offspring with superior traits and good genetic qualities, like having relatively fast, resistant growth against disease, high survival, tolerant of environmental changes, and easily cultivated [2, 3]. Chromosome set manipulation is one of the important practices for aquaculture purposes [4, 5]. Polyploidization is one of the chromosome set manipulation techniques that is being currently studied [6].
Heat shock is one of the methods used for polyploidization in fish. Heat shock methods have been widely used in several species of fish, such as goldfish [7, 8, 9], common carp [10, 11], catfish [12, 13], and Nile tilapia [6, 14, 15, 16]. Therefore, this study aimed to determine the effect of heat shock duration and initial heat shock period treatments on hatching rate, abnormality rate, long time of egg yolk absorption, and survival rate of spotted barb (*Puntius biniatus*) larvae.

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

Sperm and eggs 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 mean 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 [17] for 1.0, 1.5, and 2.0 min, respectively.

2.3. Artificial incubation of embryo

Artificial incubation of the spotted barb embryo was done 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 both the embryonic stage and the embryo abnormality.

The number of embryos that damaged or dead and hatched was calculated. The first hatched embryos were also observed. After all the embryos were hatched around 24-26 hours after fertilization (haf), the number of hatched fish larvae was calculated, including morphologically abnormal larvae. The length of egg yolk absorption and survival rate of spotted barb larvae were also calculated after 70-96 hours after hatching (hah).

2.4. Data analysis

Data were analyzed statistically use analysis of variance (ANOVA). The treatment differences were determined using Duncan’s multiple range test. The statistics are analyzed using SPSS 10 (statistical software). The significant level was determined at p<0.05.

3. Results and discussion

3.1. Results

The results showed that the spotted barb eggs have good quality and relatively uniform between treatment and control groups (p>0.05) with the fertilization rate of 82% average (Table 1). The results showed significant differences (p<0.05) between treatments of heat shock duration and initial period, and their interaction on hatching rate of spotted barb embryo, abnormality rate, length of egg yolk absorption, and survival rate of spotted barb larvae (Table 1).

The longer heat shock treatment has the lowest hatching and survival rates of spotted barb embryo as well as initial period treatment. The treatments have increase abnormality rate than control, while the length of egg yolk absorption has relatively similar between treatments and control.
yolk absorption, organ formation, and larval behavior [26, 27, 28]. The incubation temperature of embryo has an important influence in egg treatments because this parameter is more affected by temperature of incubation, such as the study before hatching [25]. In this study, length of egg yolk absorption was generally the same among hatching process disruption. Chorionase enzyme consists of pseudoceratine to soften the egg chorion as the disruption of hatching enzyme activity, called the enzyme of chorionase, causing bent tail. Larval abnormalities occurred in spotted barb larvae allegedly due to the hardening phase of treatment may cause the larvae exhibit a distorted or abnormal body, such as shortened tail, untailed or unhatched larvae [24].


during heat shock duration and initial period (age) of embryo on hatching rate happened to some species, such as common carp [10, 17]. Heat shock, duration period, and initial heat shock period treatment are species-specific, meaning that every fish species even their strain have different treatment tolerance [20]. The duration of 1.5 min is suspected as the range of egg developmental stage, resulting zygote development. The duration is also suspected of causing damage to the spindle threads served as chromosome attaching tool to the pole during the cleavage process, resulting in a high death. This is accordance with Solar et al. [21], who mentioned that the duration period of heat shock temperature caused high embryonal death. Mair [22] and Tave [23] stated that prolonged heat shock disrupted embryonal aspects and blastula phase development in fish, resulting in unhatched larvae [24].

Solar et al. [21] stated that heat shock temperature treatment with different duration of the treatment may cause the larvae exhibit a distorted or abnormal body, such as shortened tail, untailed or bent tail. Larval abnormalities occurred in spotted barb larvae allegedly due to the hardening phase of egg chorion as the disruption of hatching enzyme activity, called the enzyme of chorionase, causing hatching process disruption. Chorionase enzyme consists of pseudoceratine to soften the egg chorion before hatching [25]. In this study, length of egg yolk absorption was generally the same among treatments because this parameter is more affected by temperature of incubation, such as the study conducted by Mukti [20]. The incubation temperature of embryo has an important influence in egg yolk absorption, organ formation, and larval behavior [26, 27, 28].


discussion section: The quality of eggs and spermatozoa has an essential role in breeding activities, especially to chromosome manipulation by heat shock treatment. One of the factors used to assess the quality of both eggs and spermatozoa are calculating the fertilization rate [18]. Fertilized eggs were visible as showing bright colors. This was according to Rustidja [19], who stated that fertility egg development had transparent and clean color, which are easily distinguished by the unfertilized eggs.

The study about the effect of both heat shock duration and initial period on fertilization, hatching, and abnormality rates, length of egg yolk absorption, and survival rate of spotted barb after treated heat shock.

| Heat shock duration (min) | Initial period (mat) | FR (%) | HR (%) | AR (%) | Length of EYA (h) | SR (%) |
|--------------------------|----------------------|--------|--------|--------|-----------------|--------|
| Control                  | 3                    | 80.80±4.60 | 83.52±2.60 | 23.92±4.70 | 74.04±0.00 | 73.19±4.50b |
|                          | 4                    | 83.64±3.70 | 74.00±1.20 | 21.82±5.10 | 73.54±4.60abc | 84.10±0.70b |
|                          | 5                    | 79.91±8.30 | 70.51±1.30 | 7.34±3.60abc | 73.76±3.80abc | 79.13±4.90gh |
|                          | 3                    | 89.38±5.60 | 76.13±3.80 | 5.96±2.20  | 68.70±2.00  | 85.59±1.70b |
| 1.5                      | 4                    | 83.74±6.90 | 58.83±2.60 | 25.18±1.00d | 75.81±1.40b  | 53.13±4.20cd |
|                          | 5                    | 82.14±6.10 | 51.99±2.50 | 9.60±3.40abc | 71.64±1.30c  | 78.57±2.50b |
|                          | 3                    | 77.70±7.10 | 37.18±3.20 | 23.33±2.90d | 72.09±3.50abc | 48.05±4.90b |
| 2.0                      | 4                    | 76.92±6.90 | 32.10±2.30 | 19.70±9.20cd | 75.69±0.30c  | 56.25±5.62cd |
|                          | 5                    | 82.73±6.70 | 28.61±1.20 | 5.88±1.19b  | 70.53±1.00b  | 60.56±4.40c |
| Control                  | 5                    | 89.86±9.90 | 77.02±3.00 | 1.04±0.18  | 73.86±1.60abc | 83.33±4.40b |

Note: FR = fertilization rate, HR = hatching rate, AR = abnormality rate, EYA = egg yolk absorption, SR = survival rate. Different superscripts in the same column show no significant differences (p<0.05).

3.2. Discussion
The quality of eggs and spermatozoa has an essential role in breeding activities, especially to chromosome manipulation by heat shock treatment. One of the factors used to assess the quality of both eggs and spermatozoa are calculating the fertilization rate [18]. Fertilized eggs were visible as showing bright colors. This was according to Rustidja [19], who stated that fertility egg development had transparent and clean color, which are easily distinguished by the unfertilized eggs.

The study about the effect of both heat shock duration and initial period (age) of embryo on hatching rate happened to some species, such as common carp [10, 17]. Heat shock, duration period, and initial heat shock period treatment are species-specific, meaning that every fish species even their strain have different treatment tolerance [20]. The duration of 1.5 min is suspected as the range of egg developmental stage, resulting zygote development. The duration is also suspected of causing damage to the spindle threads served as chromosome attaching tool to the pole during the cleavage process, resulting in a high death. This is accordance with Solar et al. [21], who mentioned that the duration period of heat shock temperature caused high embryonal death. Mair [22] and Tave [23] stated that prolonged heat shock disrupted embryonal aspects and blastula phase development in fish, resulting in unhatched larvae [24].

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4. Conclusion
Treatment of heat shock duration and initial period influenced hatching, abnormality, and survival rates of spotted barb, however not length of egg yolk absorption.

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