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Effect of Temperature Shock on the Triploidization Success of Seurukan Fish (*Osteochilus vittatus*)

Kartini Eriani[1], Alfis Syahrin[2], ✉Zainal Abidin Muchlisin[2]

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[1]Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Syiah Kuala, Indonesia
[2]Department of Aquaculture, Faculty of Marine and Fisheries, Universitas Syiah Kuala, Indonesia

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Abstract

Seurukan fish (*Osteochilus vittatus*) has many advantages, besides the fish also has disadvantages which are the slow growth, so the temperature shock of triploidization technique was expected to solve the problem. The objective of the present study was to obtain an effective temperature to increase of triploidization success of *seurukan* fish (*Osteochilus vittatus*). The experimental method and completely randomized design model were used in this study. Five levels of temperature shocks at three replicates were tasted: 4°C (cold), 6°C (cold), 28°C (normal), 35°C (heat) and 37°C (heat). The sperms and eggs were fertilized in the plastic jar then a total 100 of fertilized eggs (zygotes) were taken randomly 3 minutes after fertilization and soaked in respective temperature for 90 seconds, and then incubated in incubation jars at the water temperature of 28-29°C. The results showed that the temperature shock gave the significant effect on the hatching and the success of triploidization success ($P<0.05$), but did not give the significant effect the fertility and survival rates ($P>0.05$). The triploid fish can be achieved using cold and heat shock, but the higher triploid fish was recorded at 37°C was the best temperature recommended for triploidization of *Seurukan* fish.

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✉ Correspondence Author:
Jl Teuku Nyak Arief, Kopelma Darussalam, Syiah Kuala, Aceh 2311
E-mail: muchlisinza@unsyiah.ac.id

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INTRODUCTION

Bonylip barb, *Osteochilus vittatus* or locally known as seurukan fish is originated from Southeast Asia including Indonesia. This is one of the popular freshwater fish in Indonesia, because of its taste and reasonable price. Several basic studies on bonylip barb have been reported by researchers, for example fecundity of bonylip barb (*Osteochilus vittatus Cyprinidae*) in different waters habitats (Syandri et al., 2015), *Osteochilus vittatus* and *Puntius javanicus* as an agen of biological in Maninjau Lake (Syandri H, 2004), reproduction aspects of sasau fish (Hampala sp.) and lelan fish (*Osteochilus vittatus*) in Singkarak lake (Uslichah and Syandri, 2003). However, the genetic improvement has not been conducted on this fish species. Also, fish farmers claim that the growth performance of the fish was low in captivity. Therefore, it is crucial to develop a practical biotechnology through genetic modification, especially chromosome-set manipulation to overcome this problem.

According to Muchlisin et al. (2014) seurukan fish has potency as a species target for aquaculture, and therefore the cultivation of this species has been initiated in Indonesia especially in Aceh Province. Several studies have been conducted on *O. vittatus*, for example genetic diversity (Mulyasari et al., 2010), breeding (Muchlisin et al., 2014; Adami et al., 2016), feeding ration (Asma & Muchlisin, 2016), application of union (*Allium cepa*) as a prebiotic source (Mayana et al., 2016). The principles of genetic modification method are used to increase production and quality of seed (Nurasni, 2012). Induced triploidy is the only effective method currently available for mass production of reproductively sterile *seurukan* fish (*Osteochilus vittatus*) for aquaculture.

Triploidization is a simple process of genetic technique to establish an individual with three sets of chromosomes (Risnandar, 2001). As the name implies, triploids have three sets of chromosomes in their somatic cells rather than the normal two sets (diploids). Although there are a few naturally occurring triploid species of fish that exist as all-female populations with unique reproductive strategies (Purdom, 1984), for most species triploidy is not a natural condition. Tetraploidy has played a role in the evolution of many widespread and economically important groups of fish, including salmonids (Allendorf and Thorgaard, 1984). The individuals of the triploids are sterile, and therefore the energy requirement for gonadal development are decreased and switched for growing (Lawson and Ishola, 2010). The triploid individual can be generated by preventing the release of polar body II (PB II) on the eggs; this process can be achieved by temperature shock (Hartono et al., 2013).

Study of triploidization using temperature shock technique has been performed on some fish species such as glass catfish (Alawi et al., 2009), nile tilapia (Mukti et al., 2009), goldfish (Mukti, 2005), African catfish (Nurasni, 2012; Olele & Tighiri, 2013), iridescent shark (Puji et al., 2012), yellow catfish (Emilda, 2003), basa catfish (Risnandar, 2001), grass crap (Cassani and Caton, 1985), and Atlantic salmon (Leclercq et al., 2011; Benfey, 2001; Galbreath, et al., 1994). These previous studies showed that growth performance of triploidy fish was faster than diploidy fish. Therefore, a triploidization technique is very promising to boost the growth performance of bonylip barb. This paper was the first reported in combining of the effect of heat and cool shocks on the fertilization, hatching and triploidy successful rates of bonylip barb *O. vittatus*.

This study aims to determine an effective temperature to increase triploidization success and survival rate of *seurukan* fish (*Osteochilus vittatus*). Then the results are expected to provide information of *seurukan* fish triploidization.

METHODS

The study was conducted in hatchery of Batee Iliek, Bireuen District and Research Laboratory, Biology Department, Faculty of Mathematics and Natural Sciences of Syiah Kuala University, Banda Aceh, Indonesia. The completely randomized design was used in this study. Five levels of temperature i.e. 4°C, 6°C, 28°C, 35°C, 37°C were tested with three replications.

The brood fish was injected with Ovaprim at the dosage of 0.1 ml kg^{-1} body weight for male and 0.2 ml kg^{-1} body weight for female. Then the abdomen was stripped gently 9 hours after hormone injection and the eggs and sperm were placed into the beaker (100 ml) and kept in an ice box (4°C) separately. The sperms were diluted with physiological solution at ratio 1: 20 of sperm to the extender (v/v).

Approximately 4 ml of eggs and 1 ml diluted sperm were mixed (1: 4 v/v of sperm: eggs), then two drops of tap water were added and mixed homogenously using a feather, and the sperm and eggs were left in contact for two minutes (Muchlisin et al., 2010). The fertilized eggs were taken randomly and put into the glass slides (every slide has 200 eggs); the slide was immersed into water batch at different temperature for 90

seconds. The slides were removed from the batch then incubated in the plastic jars at temperature 28 °C for 48 h.

The hatching eggs were monitored for 48 hours by interval of two hours, and the number of larvae was recorded 48 hours after incubation. The larvae of seurukan fish were reared in a basin with a water volume of 800 ml for 60 days. The larvae fed on tubifex three times a day until they reach point of satiation (ad libitum).

The successfull of triploid fish was examined based on the size of red blood cell nucleus by the fish age of 60 days. Then the fish was anesthetized in cold waters for 5 min, the tail fin was cut and the blood sample was dripped on object glass for subsequent evaluation. Then the object glass was dripped with 95% alcohol and then re-dripped with 10% Giemsa solution. Then the sample was rinsed with distilled water and dried at room temperature for 30 minutes before observing under the stereo microscope. According to Nurasni (2012), the diameter of the nucleus of red blood cell of diploid is between 9 µm - 10 µm, while 11 µm – 13 µm for triploid. In addition, the fertilization, hatching and survival rates were also analyzed. All data were subjected to analysis of variance (ANOVA), followed by the comparison of means using Duncan's multiple range test at a 95% confidence level (P = 0.05).

RESULTS AND DISCUSSION

Table 1 showed the average percentage of fertility, hatching rate, survival rate, and the success of triploidy at the shock temperature of 4 C, 6 °C, 28 °C (control), 35 °C and 37 °C. The average percentages of fertility were 84.67%, 86.67%, 84%, 80.33 % and 83%; while the hatching rate were 80.67%, 85.33%, 79.67%, 69.33%, and 67%. The survival rate respectively were 83%, 82.67%, 84%, 79.33% and 81.33%; whereas the triploidization success rate were 73.33%, 73.33%, 0%, 53.33% and 86.57%. Generally, it can be concluded that the highest result of fertility and hatching was found at 6 °C, while the triploidy success and body weight are best shown at 37 °C.

The results of ANOVA test showed that the temperature shock gave a significant effect on hatching rate and success of triploidization on seurukan fish, *O. vittatus* ($P<0.05$). However, it did not give the significant effect on the fertility and survival rates ($P>0.05$). The study showed that the highest fertility rate was found at the temperature of 6°C, but this value was not significantly different with other treatments. The hatching rate was also highest in the treatment of 6°C; this value was only significantly different with 35°C. Then the highest survival rate was recorded at 28°C, but this value was not significantly different with other treatments. In addition, the highest success of triploidization was found in the treatment of 37°C. However, this treatment was not significantly different with 4°C and 6°C. At least, the highest fish weight gain after 15 days was found at 37°C, but this treatment was not significantly different with 4°C (Table 1). The average value on the same column with different superscript is significantly different ($P <0.05$).

The results revealed that the highest fertility and hatching rates were obtained at heat shock of 6°C, and the value was decreased slightly when the temperatures were increased or decreased. It was alleged due to the temperature rising that affected the embryos metabolism, thus caused the embryos dead due to lack of oxygen supply. This speculation was supported by Fujaya (2000) who stated that the temperature increase for heat shock treatment caused an increase in metabolic rate 3 - 5 times higher. Thus, it would be increased the oxygen consumption and resulted in embryos death when oxygen availability is less.

The decline in fertility and hatching rates

Table 1. Average percentage and standard deviation (± SD) of fertility, hatching rate, survival rate, success of triploidization and weight gain of *seurukan* fish *(Osteochilus vittatus).*

Temp.	Treatment	Fertility (%)	Hatching (%)	Survival (%)	Triploidization success (%)	Weight of fish after 15 days
4°C	Cold	84.67±2.51^{a}	80.67±7.23^{b}	83.00±2.64^{a}	73.33±2.22^{bc}	1.88±0.58^{bc}
6°C	Cold	86.67±3.51^{a}	85.33±3.51^{b}	82.67±2.08^{a}	73.33±2.28^{bc}	1.70±0.42^{b}
28°C	Normal (control)	84.00±3.60^{a}	79.67±6.65^{b}	84.00±2.64^{a}	0.00±0.61^{a}	1.43±0.30^{a}
35°C	Heat	80.33±2.51^{a}	69.33±1.15^{a}	79.33±0.57^{a}	53.33±1.97^{b}	1.65±0.28^{ab}
37°C	Heat	83.00±3.00^{a}	67.00±2.00^{a}	81.33±1.52^{a}	86.67±1.90^{c}	2.09±0.62^{c}

in thermal shock probably is due to the inhibition of eggs development that caused the death of the embryo, thus reducing the average percentage of fertility and hatching. This assumption was supported by Arsianingtyas (2009) who stated that heat temperatures shock could damage the spindle thread that was formed during the process of cell division of the zygote. Also, Nurasni (2012) reported that the decrease in the hatching rate of African catfish (*Clarias gariepinus*) probably caused by a heat shock treatments that affected on enzyme activities due to the enzyme defection at high temperatures; thus the egg's cytoplasm will be broken that leads to eggs mortality.

Febrianto (2012) reported that the optimum ranges of temperature shock for better hatching of nilem fish (the local synonym name for *O. vittatus*) eggs in the triploidization process are between 23°C to 30°C (heat thermal). However, in this study we found that the best water temperature shock for seurukan fish (*O. vittatus*) eggs was 37°C (heat), but this value was not different significantly with 4°C and 6°C. Therefore, the study showed that the triploid of seurukan fish could be produced by both cold and heat shock treatments; but the fertilization, hatching and survival rates slightly higher in cold shock than in heat shock treatments. According to Risnandar (2001), the treatment of heat shock gave detrimental effect on the survival because the heat shock at the phase of meiosis II may cause damage to the membrane of the embryo. This provision can produce to an abnormal individual that led to a decrease in survival in the early life of larvae.

The observation of the triploidization success was done; by observing the size of red blood cells of the fish. The size of red blood cell of the triploid fish is larger than the common fish (diploid). Alawi et al. (2009) stated that the measurement of red blood cells had been widely used in studies determining the ploidy level of fish, because the increase of chromosomes triploid fish affect the size of red blood cells, including the cell nucleus that will be enlarged.

The study showed that the diameter of the red blood cells of seurukan diploid fish ranges from 7 µm – 9 µm (Figure 1 B), while the size of red blood cells of triploid fish ranges from 10 µm – 13 µm (Figure 1 A). According to Nurasni (2012), the diameter of the red blood cell of hybrid diploid African catfish was between 9 – 10 µm. These results indicated that the effect of temperature shock could be used in the triploid fish production of seurukan fish (*O. vittatus*).

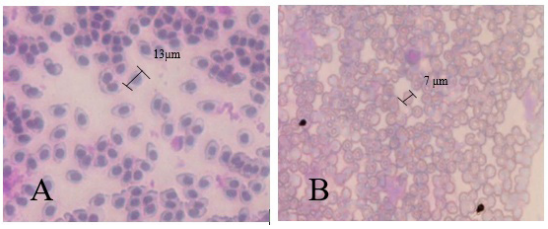

Figure 1. The red blood cells of triploid fish (A) and diploid fish (B) fish with magnification of 400X

Based on the observation of the weight gain, the averages weight of the fish in the treatment of 4°C, 6°C, 28°C, 35°C and 37°C were 1.88 g, 1.70 g, 1.43 g, 1.65 g and 2.09 g, respectively (Table 1). The results showed that the weight gain of triploid fish was higher than normal fish. According to Fujaya (2000), triploid fish is infertile, and therefore the metabolism energy can be saved and used for growing resulted in higher weight gain compared fertile fish as recorded in this study.

However, in general, the success of triploidization in the heat shock was higher than in the cold shock. This is presumably due to the cold shock temperature that gives slow propagation and resulted in many zygotes failure to form triploids. This is supported by Nuraini and Alawi (2008) who stated that low propagation of temperature would cause polar body (PB) II of being apart from the duplication of chromosomes into 3N. Thus, many zygotes formed as normal individuals.

The study indicated that the application of triploidization for seurukan fish in the heat shock at 37°C could be used to increase the growth which is slow become faster with the higher survival rate is still above 80%. It means that the triploidization applies to produce seurukan fish on fish farming can be implemented for fisherman.

This study tried to use both heat and cold shock to determine the viability, triploidy success and best temperature used on the triploidization of seurukan fish (*Osteochilus vittatus*). The results showed the best fertility and hatching rate were found at the temperature of 6°C, while the temperature of 37°C is recommended for triploidy success.

CONCLUSIONS

Induced triploidy is the only effective method currently available for mass production of seurukan fish (*Osteochilus vittatus*) sterile reproductively for aquaculture. The temperature shock

gave a significant effect on hatching rate and success of triploidization on seurukan fish, but did not give the significant effect on the fertility and survival rates. The triploid seurukan fish can be produced by heat and cold shocks, while the best temperature for triploidization of seurukan fish was at 37°C. Further research is recommended to determine the best age of zygote before shocking and the period of soaking.

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