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Effect of Cold Shock on Larval Viability and Tetraploid Percentage of Asian Redtail Catfish, *Hemibagrus nemurus* (Valenciennes, 1840)

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Abstract

This study aimed to evaluate the effects of cold shock time and temperature on larval viability and the formation of tetraploid Asian redtail catfish, *Hemibagrus nemurus* (Valenciennes, 1840). A completely randomized factorial design was used with 2 factors, shock time after fertilization (AF) which consisted of 5 levels (26^{th}, 27^{th}, 28^{th}, 29^{th}, and 30^{th} min AF) and shock temperature with 3 levels (6, 8, and 10 °C). Furthermore, each group carried out in 3 replicates, and the observed parameters including the percentage of tetraploid fish, fertilization rate, hatching, larval survival, and percentage of abnormal larvae recorded. The identification of tetraploid individuals was based on the analysis of the total nucleoli, erythrocyte size, and DNA content by flow cytometry. The results showed that cold temperature shock had an effect on hatching rate, survival rate, and the number of abnormal larvae ($p < 0.05$) but had no effect on egg fertilization rate ($p > 0.05$). The highest hatching rate was obtained in temperature shock treatment at 10 °C (60.06 ± 0.85 %), while the highest survival was obtained in temperature shock treatment at 10 °C (68.33 ± 1.38 %). Moreover, the shock time AF and temperature shock treatments influenced the formation of tetraploid the Asian redtail catfish ($p < 0.05$). Shock treatment at 28 min AF with a shock temperature of 6 °C for 3 min produced the highest tetraploid percentage (78.33 ± 1.67 %, $p < 0.05$). Tetraploid the Asian redtail catfish had a maximum number of 4 nucleoli per cell, while diploid catfish had a maximum of 2. The erythrocyte and nucleus volumes were 98.26 and 99.51 %, respectively, which were larger than those of the diploid the Asian redtail catfish. Furthermore, the DNA content in the tetraploid individuals was twice that in the diploid individuals. Thus, cold temperature shock at 6 °C for 3 min to the 28-minute-old embryos to produce a tetraploid the Asian redtail catfish is recommended.

Keywords: Cold shock, *Hemibagrus nemurus*, Induced polyploid, Tetraploid

Introduction

Asian redtail catfish, *Hemibagrus nemurus* (Valenciennes, 1840) is a freshwater species with high trade value because of its meat quality [1]. The catfish cultivation technology has been successful but is hampered by slow growth [2]. Polyploidization is an important alternative method that can be applied in aquaculture that can produce fish with desirable characteristics, such as fast growth, broad environmental tolerance, and resistance to disease [3-7]. One ploidy manipulation technique is triploidization [8-11]. Triploid fish are sterile [12], the ovaries do not develop [13] so metabolic energy is used for growth [14,15]. In addition, triploid fish are adaptable [10] and have higher meat quality [16]. The use of triploid fish in fish farming has the potential to overcome early sexual maturity [17] and control the reproductive cycle [18] so as to increase growth.

Triploid fish are produced by preventing the release of polar body II [3,19] by administering temperature shock [20], electric shock [5,9], chemicals such as cytochalasin [21], and stress [22-24]. However, the use of direct shock on embryos to produce triploid individuals has negative effects such as reduced hatchability, survival, and larval abnormalities, leading to difficulty in achieving a high proportion of triploids in the offspring [11]. In addition, direct triploid induction requires special equipment and is difficult to perform for large numbers of eggs on a commercial scale [25]. An alternative way to produce triploid fish is the formation of tetraploid (4n) fish, which are then crossed with diploid (2n) fish [11,26-28]. The induction of triploids by crossing tetraploids with diploids will produce fish with higher viability and efficiency, thereby providing potential for parental selection [29] and producing fish with fast growth and improved meat quality [30,31].

Tetraploidization is chromosome engineering to form individuals with a 4n chromosome set and is carried out by providing physical or chemical treatment to prevent the 1st cell division [32]. Tetraploid induction has been successfully developed in several fish species, including *Salvelinus fontinalis* [33], *Heterpneustes fossilis* [32], *Pangasius hypopthalmus* [34,35], *Clarias gariepinus* [36,37], *Astyanax altiparanae* [38], *Crassostrea iredalei* (Faustino) [39], and *Acipenser ruthenus* [40]. However, there has been no research regarding tetraploid induction in the Asian redtail catfish.

One method used to generate tetraploid the Asian redtail catfish is cold temperature shock. This method has been successfully used to induce tetraploid fish, such as *Clarias gariepinus* [37] and *Pangasius hypopthalmus* [41]. The success of cold shock in tetraploid species production varies widely. The best induction of tetraploid fish is determined by the shock time after egg fertilization, intensity, level, and duration [42]. Information regarding the cold temperature shock method should include timing and temperature. Therefore, this study aimed to evaluate the effect of shock timing and the appropriate cold shock temperature on larval viability and formation of tetraploid the Asian redtail catfish. The results of this study can be used as a scientific reference for producing tetraploid the Asian redtail catfish. In addition, the tetraploid the Asian redtail catfish produced can be used to produce triploid the Asian redtail catfish seeds.

Materials and methods

This study was conducted at the fish hatchery laboratory and polyfish farm field station at the Lampung State Polytechnic from 2023 to 2024. In this study, experimental protocols and animal care followed international and national ethical guidelines and were approved by the Padjadjaran University Ethics Committee Number: 257/UN6.KEP/EC/2024.

Research design

A completely randomized factorial design was used in this study with 2 factors, namely the shock time which consisted of 5 levels (26, 27, 28, 29, and 30 min AF) and shock temperature with 3 levels (6, 8, and 10 °C). In the control treatment, fertilized eggs were directly incubated in a hatching aquarium at a temperature of 28 - 29 °C without being given a cold shock. The experiments were performed in triplicate. Each replication was carried out independently and was carried out on 1 combination treatment between shock time and shock temperature.

Fish preparation

The study used broodstock the Asian redtail catfish obtained from the Polyfish Farm Laboratory at the Lampung State Polytechnic, with females and males weighing approximately 0.5 kg (2 years old) and 0.7 kg (2.5 years old), respectively. Breeding induction is carried out using ovaprim at a dose of 0.5 mL/kg body weight for female or male fish. The injection is carried out intramuscularly, that is, the injection into the muscle is carried out at the back of the dorsal fin at an angle of 45 ° [43].

The eggs and sperm were harvested after 12 h of induction by stripping process. The eggs were fertilized by mixing with sperm and then spread on a plastic filter (d-20 cm) in an aquarium at a temperature of 28 °C. A temperature shock was given to the Asian redtail catfish embryos according to each treatment at 26, 27, 28, 29 and 30 min AF with a shock temperature of 6, 8, and 10 °C. The shock treatment was carried out for 3 min [35]. For each treatment, a total of 200 to 300 embryos were used. Tetraploid induction is carried out by immersing the zygotes in a plastic filter into styrofoam filled with water at temperatures of 6, 8, and 10 °C at the ages of zygotes 26, 27, 28, 29 and 30 min AF with a shock duration according to the treatment. The decrease in water temperature is regulated by using ice cubes. After the cold shock treatment, the embryos were incubated in an aquarium at 28 - 29 °C until they hatched.

Fish rearing

The Asian redtail catfish larvae were kept in a 48-liter aquarium and provided with aeration until they were 30 days old. In the aquarium, a water heater is provided to control the water temperature at 28 - 29 °C and an aeration system was applied to maintain dissolved oxygen levels. The larvae were fed Artemia nauplii at 3 days of age until 10 days ad libitum with a frequency of 6 times a day and later fed blood worms until 30 days of age. Suction and water exchange were carried out every 2 days in larval rearing media to maintain good water quality. After 1 month of age, the fish were kept in round tarpaulin tanks with a diameter of 1.5×1.2 m^2 with a density of 50 fish m^{-2}. Fish were kept until they were 3 months old. The fish were fed commercial pellets containing 39 % protein ad libitum 3 times a day. In the maintenance media, sifting and water changes are carried out every 5 days to keep the media clean. Water quality parameters were monitored once a week. The dissolved oxygen concentration (DO), temperature of the water, pH of the water and total ammonia were measured using water quality tester. The ploidy observations were performed at the end of the maintenance period with a size ranging from 9 - 11 cm and a weight of 8 - 10 g [44].

Test parameters

The parameters of this study included tetraploid species, fertilization, hatching, larval survival, and the number of abnormal larvae. Egg fertilization was observed 6 h AF. The fertilized eggs had a transparent clear color, whereas the unfertilized eggs were white. This was calculated using the following equation [45]:

Fertilization rate = (No of fertilized egg/total number of eggs spread on a plastic filter)×100. (1)

Hatching (HR) was observed 24 h after the eggs hatched and was calculated by comparing the number of hatched eggs with the number that was fertilized [45].

Hatching rate (%) = No. of hatched larvae/fertilized eggs)×100 (2)

The observations of larval survival (SR) were carried out every 10 days until the larvae were 30 days old. The percentage of larval survival was calculated using the equation [46].

Percentage survival rate = (Survived hatchings after 3 days/total number of hatched egg)×100. (3)

Observations of larval abnormalities (AB) were carried out by observing the body morphology of 3-day-old larvae. The percentage of abnormal larvae was calculated using the following equation [47]:

Percentage larval abnormal (AB) = (Total abnormal fish/total number of larvae)×100 (4)

Ploidy assessment

The success of tetraploid induction in the Asian redtail catfish was analyzed based on the maximum number of nucleoli with silver staining [48] and erythrocyte size [4,49,50]. The results of ploidy analysis based on nucleoli and erythrocytes were verified by flow cytometry analysis using the flow cytometry method [51]. Identification of erythrocyte size commences with the preparation of blood smears. Blood was extracted from the 4-month-old the Asian redtail catfish using a 1 mL syringe in the caudal vein. The blood was subsequently applied to a clean glass slide. The slide was allowed to air dry for a few seconds, then fixed in 95 % methanol, and stained with Giemsa (10 %) for 20 min. The preparation was rinsed with double distilled water and covered with a glass coverslip. The length and width of the cells and nuclei were measured for 50 erythrocytes from each individual using a micrometer. The erythrocyte measurements are presented in **Figure 1**.

Nucleolus identification was conducted by fixing the sample using Carnoy's solution (1 acetic acid: 3 ethanol) for 60 min, with the solution being replaced every 30 min. The samples were dissociated by adding

3 drops of 50 % acetic acid to a concave glass slide for 6 - 10 s. Subsequently, the cell suspension was aspirated and transferred to a glass slide placed on a warm hot plate (45 - 55 °C) to form a circle with a diameter of 1.0 - 1.5 cm. After the preparation had dried, it was stained using silver nitrate by adding 2 drops of solution A (10 g of $AgNO_3$ dissolved in 20 mL of distilled water) and 1 drop of solution B (2 g gelatin, 50 mL water, 50 mL glycerin). The preparation was then placed in a staining chamber at a temperature of 40 - 45 °C for 20 min until it turned yellowish-brown. The nucleolus preparations were rinsed with distilled water and dried, then observed under a microscope at 400× magnification. A total of 40 fish samples were collected for each treatment. For nucleolus observation, nucleolus counting was carried out by calculating the maximum number of nucleoli per cell. Each sample was observed 450 - 550 cells, then the number of cells with 1, 2, 3, and 4 nucleoli was calculated.

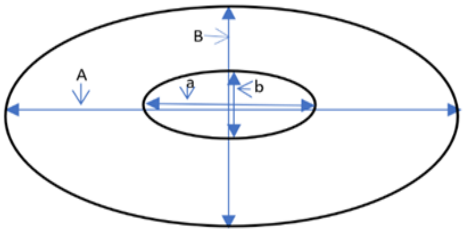

Figure 1 Measurement of erythrocytes in the Asian redtail catfish (A = Major cell axis, B = Minor cell axis, a = Major nuclear axis, b = Minor nuclear axis.

Erythrocyte area = A×B

Erythrocyte volume = 4/3×µ×(A/2)×(B/2)2

The percentage of tetraploids (PT) resulting from the treatment was determined using the following equation [35]:

The percentage of tetraploid (%) = Total tetraploid individuals/total test fish)×100. (5)

Data analysis

Data on the percentage of tetraploid, fertilization rate, hatching rate, survival rate and abnormal larvae were analyzed using the Two-Way Analysis of Variances (ANOVA) method, with Tukeys Multiple Comparison Test, using STAR (Statistical Tool for Agricultural Research) version 2.0.1. The results of the statistical analysis will show the optimal cold temperature shock for tetraploid induction.

Results and discussion

Results

The impact of cold temperature shock on the Asian redtail catfish embryos is presented in **Figure 2**, detailing fertilization rate, hatching rate, survival rate, and abnormality. Cold shock treatment did not significantly influence the fertilization rate ($p > 0.05$), which ranged from 73.00 ± 2.77 to 77.55 ± 1.21 % (**Figure 2(a)**). The timing of shock AF and the shock temperature significantly affected hatching rate and larval survival ($p < 0.05$) (**Figures 2(b)** and **2(c)**). However, no interaction ($p > 0.05$) was observed between shock timing and temperature regarding egg hatching rate and larval survival. Cold shock treatments resulted in hatching rates ranging from 49.65 ± 1.28 to 60.06 ± 0.8 %. The findings indicated an inverse relationship between treatment temperature and hatching rate. The results showed that the lower treatment temperature, the lower hatching rate. Furthermore, the hatching rate increased when cold shock was applied to older embryos.

The analysis revealed no significant interaction ($p > 0.05$) between timing of shock AF and the shock temperature on survival rates. Nevertheless, both factors independently exerted a substantial influence on survival, as evidenced in **Figure 2(c)**. Cold shock treatments yielded survival rates ranging from 53.97 ± 1.93 to 68.33 ± 1.38 %. Notably, the 10 °C temperature shock treatment demonstrated the highest survival rate (68.33 ± 1.38 %), which was statistically significant ($p < 0.05$) when compared to other treatments. The results indicated that lower treatment temperatures resulted in lower survival rates. The results also demonstrated that there was no interaction ($p > 0.05$) between the shock time and shock temperature on larval abnormalities. However, larval abnormality was significantly influenced by the shock temperature as shown in **Figure 2(d)**. The data revealed that lower shock temperatures corresponded to higher larval abnormality rates. The percentage of abnormality in the cold shock treatment group ranged from 2.11 ± 0.17 to 6.63 ± 0.41 %. The lowest level of abnormality was recorded at a temperature of 10 °C and the highest at a temperature of 6 °C.

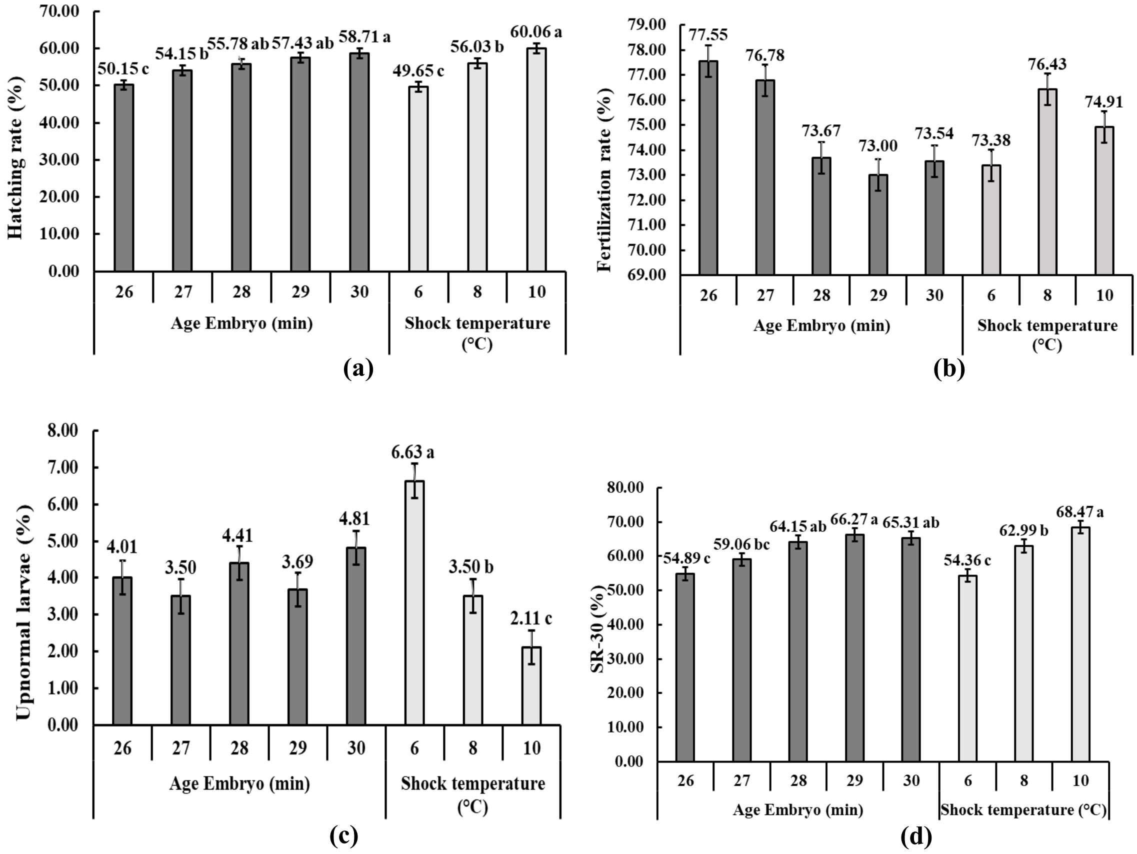

Figure 2 Fertilization rate (a), hatching rate (b), survival rate (c), and (d) abnormality of larvae subjected to cold shock treatment for 3 min at various embryo ages and shock temperatures. The error bars represent the average standard deviation (SD). The different letters above the error bar indicate a significant difference between the $p < 0.05$ treatments.

The timing of shock AF and the shock temperature treatments affect the percentage of tetraploid catfish larvae formed ($p < 0.05$). In addition, there were no tetraploid catfish individuals from the control without being given cold shock. Cold shock treatment given AF produced tetraploid catfish offspring with a range of 42.78 ± 2.06 to 72.78 ± 2.37 %. The results of the treatment showed percentage of tetraploids increased until the 28th min of cold shock, then a decrease in the percentage of tetraploid individuals occurred in the 29th and 30th min of embryo treatment. In the timing of shock AF, the highest tetraploid was obtained at the 28th min of shock (72.78 ± 2.37 %) AF. In addition, based on the shock temperature, lower shock temperature indicated an increase in the percentage of tetraploid individuals formed. The highest tetraploid fish were obtained at a shock temperature of 6 °C (61.00 ± 2.88 %) and the lowest at a shock temperature of 10 °C (52.00 ± 2.48 %). **Figure 3** shows the interaction between timing of shock AF and shock temperature on the resulting tetraploid. Cold shock treatment at 28 min AF with a shock temperature of 6 °C produced the highest tetraploid percentage of 78.33 ± 1.67 % (**Figure 3**).

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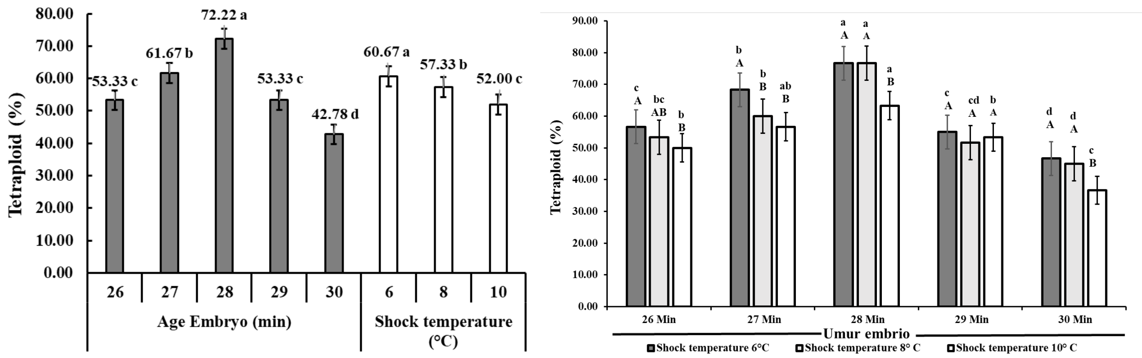
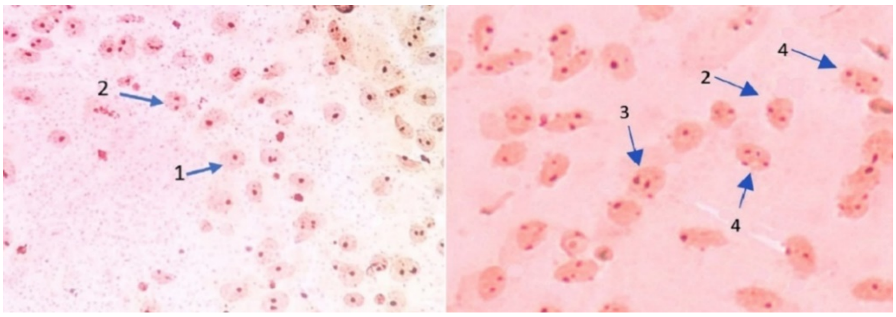


(a) **(b)**

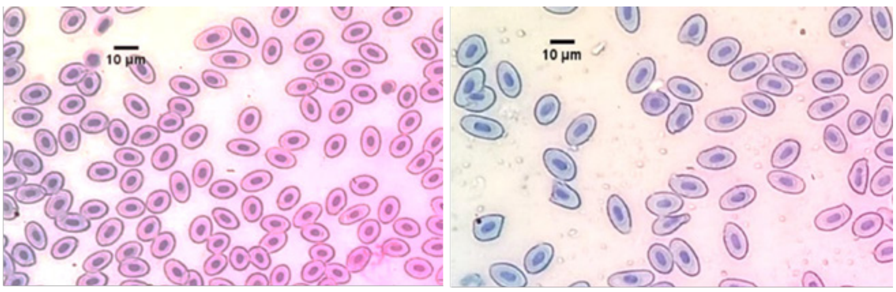
Figure 3 Effect of embryo age and cold temperature on tetraploid the Asian redtail catfish (a), interaction between treatments on the formation of tetraploid the Asian redtail catfish (b). The error bars represent the average standard deviation (SD). Different lowercase letters on the same line differ statistically by Tukey's test at 5 % probability. Different capital letters in the same column differ statistically by the Independent Samples t test at 5 % probability.

Tetraploid fish individuals were identified based on the maximum number of nucleoli per cell (**Figures 4(a)** and **4(b)**), erythrocyte size (**Figures 5(a)** and **5(b)**), and DNA content using a flow cytometry method based on single-cell size sorting (**Figure 6**). The tetraploid fish had a maximum of 4 nucleoli per cell (**Figure 4(b)**), while the diploid controls had a maximum of 2 nucleoli per cell (**Figure 4(a)**). The characteristics of tetraploid the Asian redtail catfish erythrocytes included cytoplasm and a larger nuclear size than diploids (**Table 1**). The volume of tetraploid cells was 98.26 %, which was higher than the diploid cells. Furthermore, the nuclear volume was 99.51 % larger than diploid cells. In addition, the area and volume of the cytoplasm and nucleus of tetraploids were almost double of diploids, with an increase ranging from 63 to 99 %.



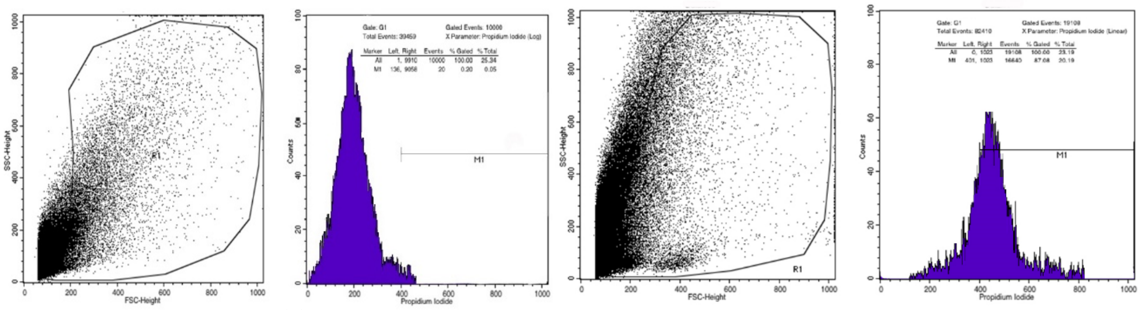
(a) **(b)**

Figure 4 The nucleolus of a diploid individual with a maximum of 2 nucleoli (a), a tetraploid individual with a maximum of 4 nucleoli (b) (arrows indicate the nucleolus).



(a) **(b)**

Figure 5 Erythrocytes of the diploid the Asian redtail catfish (a) and erythrocytes of the tetraploid the Asian redtail catfish (b). Photo used 400× magnification.



(a) **(b)**

Figure 6 Flow cytometry analysis showing the DNA histograms and scatter diagrams of propidium iodide-labeled the Asian redtail catfish: (a), diploid, (b) tetraploid. Diploid the Asian redtail catfish were used as a control for the amount of DNA. FSC-H, forward side scatter-height; SSC-H, side scatter-height.

Table 1 The size of the diploid and tetraploid the Asian redtail catfish erythrocytes.

Erythrocyte parameters	**Erythrocyte size (µm)**		**Ratio**	**Exclusive tetraploid**	**%**
	Diploid	**Tetraploid**	**(2n:4n)**	**range (µm)**	**Increment**
Cell major axis (µm)	11.29 ± 1.07	15.62 ± 1.90	1.38		38.36 %
Cell minor axis (µm)	7.29 ± 0.47	8.84 ± 1.19	1.21		21.30 %
Cell area (µm 2)	82.26 ± 8.88	123.10 ±95.00	1.50	79.41 - 206.16	49.66 %
Cell volume (µm 3)	347.85 ± 46.67	625.34 ± 118.11	1.80	450.19 - 918.97	79.77 %
Nucleus major axis (µm)	5.07 ± 1.19	7.03 ± 0.73	1.39		38.63 %
Nucleus minor axis (µm)	2.96 ± 0.55	3.72 ± 0.53	1.26		25.86 %
Nuclear area (µm 2)	15.45 ± 6.60	26.31 ± 5.33	1.70	14.22 - 43.11	70.33 %
Nuclear volume (µm 3)	31.56 ± 10.23	62.53 ± 6.16	1.98	48.29 - 99.86	98.12 %

The relative locations of different histogram peaks of fluorescence for diploid and tetraploid individuals, where tetraploid the Asian redtail catfish contained 2-fold higher DNA content than diploid catfish. The peak in channel 200 indicated diploid the Asian redtail catfish, whereas channel 400 showed tetraploids (**Figure 6**).

The water quality parameters measured during the experiment were within the appropriate range, specifically for water temperature (28.1 - 30.8 °C), DO (4.2 - 5.5 mg/L), pH (7.1 - 8.3), total ammonia (0.1 - 0.3 mg-N/L). This parameter has been reported to have no effect on the presence of this the Asian redtail catfish.

Discussion

The cold shock treatment in the 1st mitotic phase, administered 26 to 30 min AF could prevent the 1st cell division [40] leading to the formation of tetraploid individuals. This was in accordance with Wu *et al*. [19] and Hartono *et al*. [35] who stated that the formation of tetraploid fish was achieved by administering a temperature shock in mitosis phase 1. The age of the embryo that produced the highest tetraploid was 28 min, at temperatures of 6 °C. At 28 min, it was observed AF, it was the peak period for the 1st mitosis. The effectiveness of the temperature shock decreased after the 1st mitotic peak in tetraploid formation because the 1st division was completed [52]. However, after the 1st peak of mitosis, the cell entered anaphase and telophase with separated chromosomes, and cold temperature shock no longer had a significant impact on the formation of tetraploids. Furthermore, variations in the percentage of tetraploids formed, which did not reach 100 %, could be caused by differences in embryo development within a population [4,5]. During the shock period, there may be eggs have not yet entered or even passed the 1st cell division phase, leading to an uneven distribution of the shock effect on the eggs. In addition, the temperature shock has an effect on the formation of tetraploid fish. However, the effectiveness of tetraploid induction is not only determined by the shock temperature, but is also affected by the temperature before the shock and the shock temperature. The higher the shock temperature interval with the egg incubation temperature, the more successful the tetraploid induction issuance. This is evidenced by research that shows that the lower the shock temperature, the more effective it is in producing tetraploid fish. This is in accordance with Martin *et al*. [53] who showed that the higher the distance between the shock temperature and the incubation temperature of the eggs, the higher the tetraploids. A shock duration of 3 min successfully induced tetraploid fish, but not with 100 % accuracy. According to Alvarenga *et al.* [54], a longer shock period is more efficient for polyploid formation. The timing of cold shock administration should be accurate to maximize the formation of tetraploid fish while minimizing induction failure. However, a longer shock duration could potentially lead to a lower larval survival rate than that observed in this study. Differences in the temperature shocks had an impact on the appearance of tetraploid fish, thereby showing a tendency increase the percentage of formation at lower shock temperatures [54].

The results of other studies have shown that cold shock produced tetraploid fish with varying degrees of success. Research by Syahril *et al*. [41] on *Pangasius hypopthalmus* produced 70 % tetraploids with a cold shock at 8 °C for 29 min. The allocation rate of tetraploid *Clarias gariepinus* was 37.12 % [37] with cold shock at 5 °C for 5 min. Cold shock of 1 °C produced 31 % tetraploid larvae *Mercenaria mercenaria* [52]. Tetraploid induction in *P. monodons* with shock temperatures between –2 - 1 °C produces tetraploids between 14.9 and 24.6 % [55]. The success of tetraploid fish induction is determined by factors such as the shock time after egg fertilization, shock level, shock intensity, and shock duration [42,48]. In addition, the interaction between temperature before and during the shock, the timing of the shock and the duration of the shock are essential to maximize the effectiveness of the induction process.

Cold temperature shock to the embryo AF did not affect fertilization because the shock occurred after the fertilization process. The effects of the shock were observed after the shock and not in the process before the shock. This study showed a trend towards decreased hatching and survival with lower shock temperatures and an increase in abnormal larvae. These results are in line with those of [56], who reported that cold temperature shock on polyploidy affected hatching, survival, and increased abnormal cell division during embryogenesis. The difference between shock and rearing temperature is very important because it defines the effect of shock on embryonic and larval development, including abnormal division and abnormalities [40].

Disruption of cell division [57] and cold temperature shock during mitosis phase 1 can also cause physical damage to the structure of the plasma membrane [58]. In addition, disruption of embryonic development, such as protein denaturation and enzyme dysfunction, can trigger a stress response in the developing embryo [59], thereby affecting the viability, hatching, and survival of treated eggs [20,37]. This can be observed from the tendency for a decrease in the degree of hatching and survival of larvae, as well as an increase in the total number of abnormal larvae at lower shock temperatures.

Egg hatching and larval survival after cold temperature shock were still better than those of several other tetraploid treatments. The yellow-tailed tetra fish (*Astyanax altiparanae*) induced by heat shock resulted in 19.1 % egg hatching [38]. Heat shock in the fish *Heteropnestus fossilis* resulted in a larval survival rate of 76 % [32]. Induction of tetraploid catfish through temperature shock resulted in a survival rate of 40 % [48]. Myers *et al.* [60] also observed up to 8.3 % survival of tetraploid tilapia, using hydrostatic pressure and cold shock. Heat shock in rainbow trout (*Oncorhynchus mykiss*, Walbaum, 1792) resulted in hatching and larval survival of 55.33 ± 1.45 and 67.33 ± 1.20 % [61]. This suggests that cold shock is an effective method for producing polyploid fish, has a smaller negative effect on embryo and larval development compared to heat shock [62].

Furthermore, larval abnormalities resulting from cold temperature shock were lower than those resulting from heat shock treatments in catfish, ranging from 12.36 to 27.60 % [63]. A higher level of larval abnormalities was observed by [47] in spotted barb (*Puntius binotatus*), which were treated with heat shock before the 1st mitosis, resulting in abnormalities of 5.88 to 25.18 %. The use of cold shock had a less negative impact on larval survival rates than heat shock treatment. Shock treatment has an impact on increasing abnormal larvae caused by damage to the embryonic membrane [63]. In addition, abnormalities in fish are caused by temperature shocks causing a pair of homologous chromosomes to be unable to separate during mitosis. This results in 1 gamete receiving 2 types of the same chromosomes but the other gamete does not receive chromosomes, so if 1 gamete deviates and then unites with a normal gamete during fertilization, it will produce offspring with an abnormal number of chromosomes. If the organism is able to survive, it will show a number of symptoms caused by the abnormal number of genes located on the additional or missing chromosomes. The low survival of tetraploid fish is caused by mosaicism, aneuploidy, cytological errors, high homozygosity, and deterioration of cell surface quality [64]. However, tetraploid status itself does not increase larval mortality, although shock is a major factor contributing to low survival [61].

In this study, we used nucleolus identification (NOR) and erythrocyte size, which were verified by flow cytometry. Identification of the Nucleolar Origin Region (NOR) and erythrocyte size is an easy, fast, and inexpensive option for identifying ploidy without compromising fish [50]. This is because the sizes of the cell and nucleus are directly related to the size of the genome [65]. The results indicated that there was a difference in the size of the erythrocytes of tetraploid and diploid the Asian redtail catfish. The erythrocyte volume of tetraploids was nearly twice as large (ratio 1:1.98) compared to that of diploids. This was in line with the results of [44] and [37], who showed that there was a significant increase in erythrocyte volume in tetraploid fish compared to diploid fish. Furthermore, tetraploid blood cells have a larger size and volumetric ratio of erythrocytes than diploids [37], indicating that tetraploid induction will correct differences in the cytoplasmic nuclear ratio [33]. Changes in the erythrocyte size ratio are a consequence of an increase in the nucleus and cell volume due to an increase in genetic material [66]. Determining the exclusive tetraploid range in tetraploid the Asian redtail catfish can be used to quickly differentiate ploidy levels in populations. The erythrocytes of tetraploid fish in the tetraploid range reached 100 %, but there was an overlap with the exclusive tetraploid range of diploid the Asian redtail catfish. Therefore, the determination of the exclusive range takes into account the diploid, triploid and tetraploid erythrocytes of the Asian redtail catfish so that the exclusive range can be used to determine the ploidy of the Asian redtail catfish in the laboratory or in the field with high accuracy. The ploidy identification used was based on the nucleolus (NOR) and erythrocyte size, which was confirmed by flow cytometry analysis, providing a high level of accuracy in ploidy determination. In addition, the 3 methods used did not require killing the sample fish, thereby increasing the potential for tetraploid fish.

The results of this study were obtained using tetraploid the Asian redtail catfish. Tetraploids show high potential for evaluating the fertility of prospective broodstocks. In addition, tetraploid broodstocks can be used to produce triploid fish seeds. Tetraploids through cold shock are the 1st step to produce tetraploid fish broodstocks. Furthermore, the production of tetraploid broodstock for triploid fish production is carried out by crossing tetraploid fish resulting from cold temperature shock. This is to suppress larval abnormalities and the negative impact of shock in tetraploid fish production.

Conclusions

In conclusion, this study showed the effectiveness of cold temperature shock in the induction of tetraploidy in the Asian redtail catfish. A 3-minute cold shock was found to be best performed when the embryo was 28 min old with a shock temperature of 6 °C (78.33 ± 1.67 %). Furthermore, cold shock treatment reduced the hatching rate and survival of larvae but increased the number of abnormal larvae. The total number of fish obtained was large enough for further evaluation. Although the best cold temperature shock treatment has been obtained in producing tetraploid the Asian redtail catfish, the success of induction of tetraploid the Asian redtail catfish can be further improved by standardizing the shock temperature and duration.

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