

Induction of Triploidy by Cold Shock in Indian catfish, *Heteropneustes fossilis* (Bloch)

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Abstract

Indian catfish (*Heteropneustes fossilis*, Heteropneustidae) is a highly nutritious fish with a comparatively slow growth rate compared to other popular Indian fishes. Triploids are expected to show higher growth potential due to sterility and reduced gonadal development. In an attempt to improve growth potential in this catfish, activated eggs were subjected to cold shock at 4°C for different trial times (10, 20, 30 and 40 minutes) starting at 2 minutes post-fertilization. The optimum duration for cold shock was found to be 30 minutes. Triploids (3n=84) were distinguished from diploids (2n=56) by micromorphological measurements of erythrocytes and chromosome counts.

Introduction

Indian catfish (*Heteropneustes fossilis* Bloch, Heteropneustidae), commonly known as Singi fish, is highly regarded for food due to its high protein (22.8%), low fat (0.6%) and high iron content (226 mg·100 g⁻¹ tissue) (Anon. 1982). One major constraint in the culture of this fish is poor growth rate relative to other popular edible fish, especially carp. In order to enhance growth in *H. fossilis*, we attempted to induce triploidy by cold shock. Triploids are generally sterile due to irregular meiotic division of chromosomes, resulting in reduced gonadal development and aneuploid gametes. In such fish, therefore, energy consumption for sexual maturation may be avoided, and more biological effort directed towards improving flesh quality and somatic growth.

Induction of triploidy has been achieved in many fish species by treatment designed to block the second meiotic division or to prevent extrusion of the second polar body of the egg. Methods used to date include: exposing fertilized

eggs to temperature shock, heat or cold shock (Thorgaard 1983), hydrostatic pressure shock (Piferrer et al. 1994) and chemicals such as colchicine (Smith and Lemoine 1979), cytochalasin B (Refstie et al. 1977), nitrous oxide (Johnstone et al. 1989), and high pH multiplied by high calcium (Ueda et al. 1988). Cold shock in general has been most successful in warmwater fishes (Thorgaard and Gall 1979). However, heat shock was 100% effective in tilapia, *Oreochromis mossambicus* (Varadaraj and Pandian 1988). Among catfishes, triploidy has been induced successfully in African catfish, *Clarias macrocephalus* (Vajaratpimol and Pewnim 1990), *C. batrachus* (Manickam 1991) and European catfish, *Silurus glanis* (Krasznai and Marian 1986).

The present investigation was undertaken to induce triploidy in *H. fossilis* using cold shock. This is the first attempt to induce triploidy in Indian catfish (*H. fossilis*). Nuclear and cellular size of somatic cells in triploids are increased to accommodate the increased amount of nuclear DNA while maintaining a normal ratio of nuclear to cytoplasmic volume. These changes have been clearly demonstrated using erythrocytes. Size of erythrocytes and their nuclei have been used by many workers to reliably identify triploids (Purdom 1993). Triploids have three sets of chromosomes in every somatic cell in comparison to two chromosome sets in diploids. Therefore, chromosome analysis is the most precise and reliable method for detection of triploids. In this work, success of triploid induction was detected using both above-mentioned techniques.

Materials and Methods

Induced Breeding

Gametes of parental fish were obtained using the procedure outlined by Sarkar et al. (1979). Mature male and female fish (*H. fossilis* Bloch) weighing 150 ± 10 g were purchased from a local market and kept for 2 d under laboratory conditions and fed with *Tubifex tubifex* supplied *ad libitum*. Female fish were injected with 150 IU of human chorionic gonadotrophin (Purbergen, Uni-Sankyo Limited, India) to induce ovulation 14-16 h before stripping. Male fish were dissected and their testes taken out, washed, then macerated in fish physiological saline (0.6% NaCl). The brownish eggs were collected in plastic containers by gentle stripping. Sperm was pipetted out using a pasteur pipette, and mixed with the egg mass. A small amount of tapwater was added after 2 minutes and the fertilized eggs were divided into five different batches, each containing 350 ± 10 activated eggs.

Treatments

In a pilot study, 4°C was found to be the most appropriate temperature for inducing triploidy. Freshly fertilized eggs were exposed to 4°C for 10, 20, 30 and 40 minutes, and one batch was kept as a control at room temperature without cold shock. Each egg batch contained 350 ± 10 activated eggs. Treated

eggs were then incubated at $26\pm 1^{\circ}\text{C}$ under running water in the same conditions as control eggs. Fully formed larvae hatched after 24-26 h post-fertilization. Hatchlings were counted and hatch rate was calculated as the relative percentage of initial eggs incubated.

Rearing of Fry

Four treated and one control batch of larvae were reared separately in plastic trays. The total yolk sac was fully absorbed in 4 d. After the yolk was fully absorbed, hatchlings were fed chopped pieces of *Tubifex tubifex* during the first 15 d post-hatchling, and intact worms, *ad libitum*, thereafter. Temperature and photoperiod in the rearing room was kept constant ($26\pm 1^{\circ}\text{C}$, 8L:16D).

Determination of Ploidy

Ploidy was determined in 30 fish, taken randomly, in four treated batches and the control batch by micromerement of erythrocytes and their nuclei and chromosome counts from metaphase spreads. Diameter of 20 erythrocytes in each fish and their nuclei were measured with an ocular micrometer using Giemsa-stained preparations. Metaphase spreads of chromosomes were prepared from fish fin epithelium following the method of Denton and Howell (1969). Unpaired t-tests were used to determine significant differences in percentage of triploidy and hatchability in treated-untreated batches.

Results

The percentage of successful triploid induction of activated eggs in five treatments are presented in Table 1. No triploid fish was detected in the control, 10- or 20-minute cold-shocked batches. Triploids were observed in 30- and 40-minute cold-shocked groups. However, percentage triploidy was not significantly different between the 30- and 40-minute batches and was 94.13% in the 30-minute cold-shocked group. Hatching rate was reduced significantly ($P<0.01$) in the 40-minute cold-shocked batch (63.5%) compared to the 30-minute cold-shocked group (71.79%). Mean hatchability reduced with increased duration of cold shock between control, 10- and 20-minute cold shock groups. However, a decrease in the hatching rate between control and the 10-minute shock group, and between the 10- and 20-minute cold-shocked group were not significant at 5% level of significance.

Micromerements of total erythrocyte and nuclei dimensions, and chromosome analyses of metaphase spreads prepared from fish fin epithelium in cold shock groups at 30- and 40-minute durations confirmed induction of triploidy in both groups. Mean diameters of erythrocytes and their nuclei in triploid condition were 1.57 times greater than that of diploid individuals ($P<0.001$, Figs.1 and 2). Chromosome number in the triploid condition was 84, whereas normal diploids in the control group had 56 chromosomes (Fig. 3).

Table 1. Percentage (mean $\pm$ SE) of triploids and hatchability in fertilized eggs subjected to cold shock at 4°C for various durations. (Data represents mean of three replicates)

Duration of cold shock (minutes)	Triploids (%)	Hatchability (%)
0 ^a	0	82.75 $\pm$ 1.40
10	0	79.57 $\pm$ 1.38
20	0	72.30 $\pm$ 2.69
30	94.13 $\pm$ 1.67	71.79 $\pm$ 1.04
40	91.70 $\pm$ 2.00	63.50 $\pm$ 1.10

^aFertilized eggs in the control group.

Total number of eggs in each group was 350 $\pm$ 10.

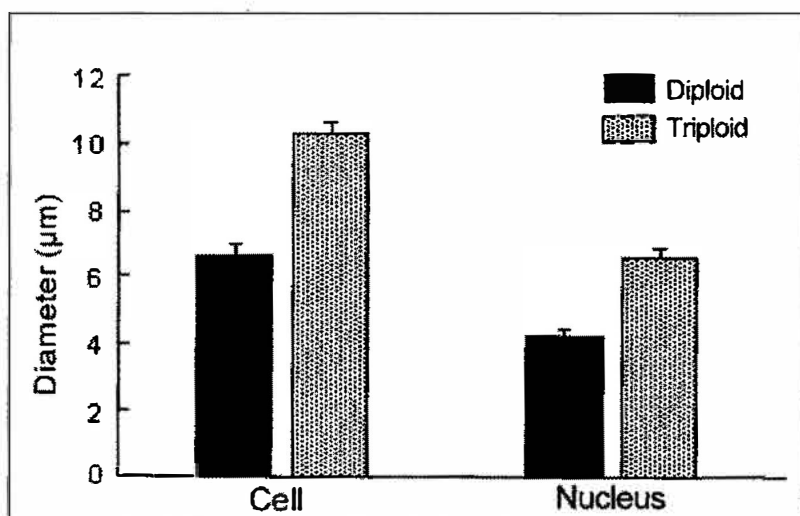


Fig. 1. Histograms showing differences in cellular and nuclear diameters of erythrocytes between diploid and triploid catfish (*H. fossilis*).

Fig. 2. Photomicrographs of erythrocytes obtained from diploid (2n) and triploid (3n) catfish (*H. fossilis*) x 500.





Fig. 3. Photomicrographs of metaphase chromosomes prepared from diploid ($2n=56$) and triploid ($3n=84$) catfish (*H. fossilis*) $\times$ 1200.

Discussion

Although triploidy has been induced in various species of catfish (Wolters et al. 1981; Krasznai and Marian 1986; Richter et al. 1987; Vajaratpimol and Pewnim 1990; Manickam 1991), no information regarding induction of triploidy is available for the commercially important catfish species, *H. fossilis*. This study is the first to undertake successful induction of triploidy in Indian catfish (*H. fossilis*). Under experimental conditions, the highest success with triploid induction (94.13%) was induced in catfish following a cold shock at 4°C, 2 minutes after egg activation. Effective durations of cold shock for obtaining more than 90% triploid fish were 30- and 40-minutes exposure. The highest hatching rate (71.79%), however, was achieved after 30 minutes of exposure to cold shock. A significant decrease ($P<0.01$) in hatchability in the 40-minute exposure group may be due to the lethal effect of prolonged exposure to cold. Results indicate that the second meiotic division and the extrusion of the second polar body in *H. fossilis* took place quickly (within 2 minutes) after fertilization. Similar results have been reported for *Clarias macrocephalus* (Vajaratpimol and Pewnim 1990).

The size of erythrocytes and their nuclei can be used to identify ploidy. Cell and nuclear diameters of erythrocytes in diploid and triploid individuals were significantly different ($P<0.001$). These results agree with previous studies by other workers dealing with other fish species (Richter et al. 1987; Purdom 1993).

Triploid *H. fossilis* showed better growth rates than normal diploid individuals under controlled laboratory conditions. Triploid males exceeded body weights of respective controls on average by 38.9%, whereas triploid females displayed an even higher increase in body weight compared to diploid full-sibs (143.5%) at the end of a 1-year growth cycle (Tiwarly et al., unpubl. data).

The growth performance of triploids is a critical factor in determining their utility in aquaculture. Since the growth process in fish is controlled by a variety of environmental factors, further observation on growth of triploids under field conditions is in progress. *H. fossilis* is widely distributed in India as well as in other Asian countries, including Sri Lanka, Myanmar and China. Therefore, triploids of this catfish may be of great value to fish farmers in many other Asian countries as well.

Acknowledgement

B.K. Tiwary and R. Kirubakaran are grateful to the Council of Scientific and Industrial Research, Government of India, New Delhi, for financial support in the form of a Research Fellowship (NET) and Research Associateship, respectively.

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