TRIPLOIDY INDUCTION IN Heterobranchus longifilis (FAMILY: CLARIIDAE) BY COLD SHOCK

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ABSTRACT

Triploidy was induced in African Catfish (*Heterobranchus longifilis*) by cold shocking activated eggs at 5°C for forty minutes starting 3 - 4 minutes after fertilization.

Triploidy was confirmed from mitotic chromosomes prepared from embryo which showed 100% triploidy in the cold shocked treatment and 100% diploidy in the control treatment.

INTRODUCTION

One of the current methods of improving growth performance of aquacultural species is through biotechnology approach. This could be through hybridization, chromosome manipulation, gene manipulation etc. Chromosome manipulation may be in form of Triploidy induction. This method involved the inhibition of extrusion of the second polar body during the second meiotic division (Thorgard, 1983).

Many authors have published instances of successful triploidy induction by cold shock in different species in both freshwater and marine species (Chao *et al.*, 1986; Tave, 1994; Manickam, 1990; Olufeagba *et al;* 1996).

H. longifilis potentials as a good aquacultural species has been described (Teugels, *et al.* 1990). The karyomorphology is also known (Olufeagba in press) but a thorough study of the early survival and growth of triploid compared with the control has not been reported. Therefore, in this study, *H. longifilis* triploids was induced by applying cold shock at 5°C for forty minutes in cold chamber on 3-4 minutes old artificially activated eggs.

MATERIALS AND METHOD

Breeders weighing between 250-500 grams were collected from the wild from Anambra State of Nigeria (5° latitude). They were transported to National Institute for Freshwater Fisheries Research (NIFFR)New Bussa 10m x 10m x 1.5m^3 outdoor concrete tanks for them to acclimatize. They were fed with 40% crude protein NIFFR feed. During breeding, male with protruded reddish genitalia was choosing and a female that released free eggs on gentle strolling of fingers on the belly.

The breeders were injected with one dose of 0.5 ml per kilogram of fish. The latency period before stripping was 15 hours at 26°C (±1). After fifteen hours, the male was sacrificed and the testes were removed. The eggs were hand-stripped and were fertilized with the sperm from the testes.

TRIPLOIDY INDUCTION

Cold shock treatment of the fertilized egg was done inside a cold chamber maintained at 5°C for 40 minutes. The fertilized eggs were distributed into four beakers. Kakabans were put in the beakers for the eggs to adhere to them. Cold shocking started 4 minutes after fertilization.

One 60cm x 30cm x 30cm^3 aquaria served as control as the eggs were allowed to develop normally under room temperature (25°C ±1).
After 40 minutes of cold treatment, the eggs were transferred to 60cm x 30cm x 30cm^3 aquaria with aerated water at room temperature.

RESULTS

Percentage hatchability was calculated based on the hatchlings at twenty four hours. Survival rate was monitored for fourteen days and analysis of variance was used to find significance difference at 5% between the control and triploid survival. After two weeks of monitoring in glass aquaria, the fry were transferred to net hapa fixed in 4mx4m x 1.2 m^3 earthen pond. Fish were fed once daily with 40% crude protein feed. Fry have access to natural zooplankton, as the pond were regularly fertilized with chicken droppings.

ASSESSMENT OF PLOIDY LEVEL

Triploidy induction was confirmed from 12 hours old fry that was allowed to swim in 0.001% colchicine and 0.5% cobalt chloride solution for twelve hours, followed by two hours swimming in distilled water. The fry were then removed and preserved in fixative made up of one part glacial acetic acid and three parts analytical ethanol. The fixed hatchlings were kept in the refrigerator until use.

Trunk regions of 2-3 hatchlings were minced with fine tipped forceps for one minute to form cell suspension in a depression slide. 2-3 drops of the suspension was then placed on warmed slides and allowed to dry on slide warmer maintained at 40-45°C. The slides were stained for 30-40 minutes with F.L.P. orcein stain (formic acid, lactic acid, propionic acid). The stain was rinsed off and dried again. The slides were observed using a Zeiss binocular microscope equipped with a camera. 50 well spread metaphase were examined in both the triploid and control cells. The mean number of the chromosome count was taken as the Chromosome number.

Well spread metaphase spread were photographed and the negatives were developed and printed.

Ninety fry each in the control and triploid treatments were distributed in 3 aquaria (30 fry/aquaria) to monitor daily survival for two weeks, after which the pooled weight and average length were determined. Fry were fed with zooplankton collected from NIFFR natural fish food production unit.

The results of the hatchability are shown in table 1. The cold shock eggs had 48.2% hatchability, while 93.2% hatchability was recorded in the control experiment.

Table 1: Percentage fertilization, hatchability and survival in *Heterobranchus longifilis*.

Treatments	Number of Eggs (±10)	Number of Fertilized Eggs (±10)	Number of Hatchlings (± 10)	Number of abnormal embryo
Triploid	350	320 (91.4)	154 (48.2)*	7 (4.5)**
Control	350	315 (90)	293 (93.2)*	2 (0.6)**

*Percentage hatchability in relation to number of fertilized eggs
** Percentage abnormal embryo

In the control treatment, the mean number of chromosome was 50 number (Plate 1A) while in triploids, 75 was the average number of chromosomes (Plate 1B).

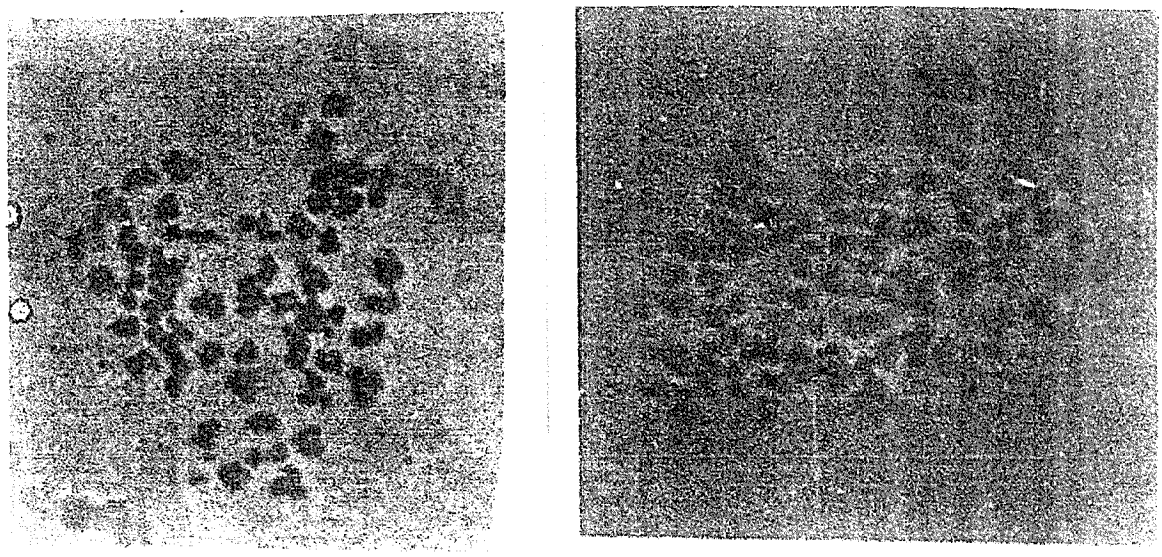
Less than 5% of the triploid, and less than 1% of the control treatments developed some abnormal characteristics. These include short or curved tail, distorted. Yolk sack and microcephalic. These embryo died within three days of hatching.

The daily survival rate is shown in Table 2, and the mean length (cm) and mean weight (grams) are shown in Figure 2. After 2 weeks, the mean weight for the triploids was 0.043gm while for control it was 0,039 gms. The mean length for Triploids was 1.7cm, while for control, it was 1.5cm.

The number of survival in Triploid was 74 while, in the control, 80 survival was recorded after 2 weeks. There was no significant different between the survival in control and Triploid experiment. (0.05% cal. F value = 3.416).

Table 2: **Survival, Mean length, and Weight of diploid and triploid *Heterobranchus longifilis* reared in aquaria tanks.**

Treatments	Weight (gm)	Length (cm)	Percentage Survival
Triploid	0.061	1.8	66.1
Diploid	0.041	1.5	91.6



A B

Plate 1: (a) Diploid metaphase chromosome in *Heterobranchus longifilis* 2n = 50
(b) Triploid metaphase Chromosome in *H. longifilis* 3n = 75 (mag x 3260)

DISCUSSION

Triploidy was successfully induced in catfish eggs exposed to cold shock at 5°C for a duration of 40 minutes as shown in plate 1b. This experiment still continue to increase triploid yield from cold shocking of eggs.

The triploid fish were as viable as the diploid after hatching. This was clearly shown in the Fishers (1948) analysis of variance test which confirmed no significant difference at both 5% and 10% significant levels in the survival of diploid and triploid after hatching. The low percentage hatchability is in agreement with previous authors (Hervai *et al* 1980; Manickam, 1991; Linhart *et al*; 1991; Olufeagba *et al* 1996).

The goal of triploid induction is to increase the growth rate above the diploid control (normal). This has been demonstrated due to lack of sexual maturation in sterile triploid (Valenti, 1975; Purdom, 1976). This is expected to be more pronounced after sexual maturity, however, this has been shown in this experiment where the mean weight and mean length of triploid were higher than normal group (Table 2). No work on early growth of Triploid has been published.

The fry are being raised to maturity to record data on growth, feed efficiency and gonado somatic index under earthen condition. Any desirable feature gained from triploid *H. longifilis* will go a long way to increase catfish production.

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