INDUCTION OF ARTIFICIAL MEIOTIC GYNOGENESIS WITH ULTRAVIOLET RAYS IN THE AFRICAN CATFISH, *CLARIAS ANGUILLARIS*

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ABSTRACT

Artificial gynogenesis was induced in *Clarias anguillaris* by fertilizing the eggs with UV irradiated sperm and by administration of cold shock, 4 minutes after fertilization at 4°C for 4 minutes. Yields of 6.2% diploid gynogenetic hatchlings and 79.90% normal diploid (Control) hatchlings were obtained at hatching. Diploidy inductions was confirmed by diploid metaphase chromosomes in the diploid gynogens (2n=54). Survival at about three months was 86.7% for diploid gynogens and 66.7% for normal diploid (Control). Mean weight gain per day of 0.4 and 0.235g were recorded for diploid gynogens and control respectively in 42 days.

INTRODUCTION

Gynogenesis is a type of parthenogenesis triggered by a genetically inert spermatozoa. For many fish species, including diploid loach (Suzuki *et al.*, 1985); Common carp (Komen *et al.*, (1988); Salmonids (Onozato and Yamaha 1993) and Tilapia (Chourrout and Itskovich 1983) gynogens induced by activating eggs with gamma or UV irradiated spermatozoa do not survive beyond early embryonic stages because of the expression of the so-called haploid syndrome. In order to overcome this problem, the resultant haploid eggs have to be made diploid by suppression of the second meiotic division (retention of the second polar body) or of the first mitotic division. Viable diploid gynogenesis is a very promising technique for accelerating the rate of inbreeding or production of clones. All female progenies are expected to be produced with the use of this chromosome engineering procedure.

The study was therefore designed to produce highly productive inbred stains of *Clarias anguillaris*

MATERIAL AND METHODS

Egg and Sperm Specimens

Ripe eggs were hand-stripped from female, (230-500g) injected with 4mg/kg carp pituitary hormone in a single full doze after a latency period of about 12 hours. Spermatozoa of the male were obtained by removing testes of injected males (4mg/kg carp pituitary hormone) for the same latency period with the females.

Sperm Irradiation

At least two testes were macerated in a petri dish. Spermatozoa were spread about 1mm thick in a petri dish put 7cm under the UV light source germicidal lamp (2454mm) for 1 minute. The petri dish was agitated mechanically to ensure even irradiation.

Control

About 1,500 eggs were fertilized with non-irradiated spermatozoa (about 0.5ml) and allowed to proceed with incubation at a temperature of 25°C.

Experimental Cross and Chromosome Manipulation

Gynogenetic experiments were designed to produce meiotic gynogens as follows. Batches of eggs activated with UV irradiated spermatozoa were cold shocked at 4°C for 40 minutes, 4 minutes after fertilization. Two sets of control were designed. One set consisted of eggs activated with UV-irradiated spermatozoa (about 0.5ml) without further manipulation. Another set consisted of fertilizing eggs with non-irradiated spermatozoa.

All experiments as described above were carried out in duplicate or triplicate at a temperature of 25°C - 26°C and a pH of 6.53 to 7.17. All batches of eggs were incubated after each treatment in 60 x 30 x 30 cm^3 glass aquaria containing well aerated water provided with egg collectors.

Chromosome Preparation and Observation

About 22 hours after fertilization, randomly selected developing eggs in control crosses, and gynogenetic cross, UV + cold chock (UVCS), were fixed directly in freshly prepared fixative consisting of 3 parts of ethanol and 1 part of glacial acetic acid for a minimum of about three minutes or kept in a refrigerator until use. Slides of chromosomes were prepared according to Kligerman and Bloom (1997) and stained with F.L.P. (Formic acid, Lactic acid and Propionic acid orcein. Slides viewed under the light microscope for chromosomes at metaphase. Well spread metaphases were selected from photomicrography. Prints were made of the negatives of the film for chromosome analysis.

Feeding Trials

At feeding start (four days after hatching) the fry were fed with zooplankton consisting mainly of *Moina duba* cultured by the limnology programme of the Institute *ad libitum* for a period of about 7 weeks. Rearing temperature was 26°C at a pH 6.53 to pH 7.17. At 7 weeks after hatching, 15 fingerlings from each treatment were selected randomly and placed in separate aquaria. The fish were fed with 5% body weight of 40% crude protein feed prepared by the Fish Technology Programme of the Institute. The experiment lasted 42 days. Mean fresh body weight per aquarium was determined biweekly. The record of fish mortality was kept during the period.

RESULTS AND DISCUSSION

Normal diploid control hatchlings were observed at 22 hours 18 minutes after fertilization. Hatchlings of UV haploid control and diploid gynogens (UV + cold shock treated embryos) were observed at 22 hours 49 minutes after fertilization. Over 75% of the fertilized eggs hatched in both control groups whereas only 6.2% of fertilized eggs hatched in the treatment that received a combination of UV and cold shock. About 30.1% of hatchlings in the haploid control group were deformed and died within the period of yolk absorption (first 4 days). No mortality was recorded for the second control group. In the diploid gynogenetic group, only 15.4% of the hatchlings died during the four - day yolk absorption period. The effect of the UV dosage, cold shock and the expression of the haploid syndrome are the likely

causes of these high deformity and mortality of hatchlings. This results agrees with the findings of Volckaert *et al.*, (1994) who reported 57-93% mortality of the haploid phenotype of *C. gariepinus* within 24 hours.

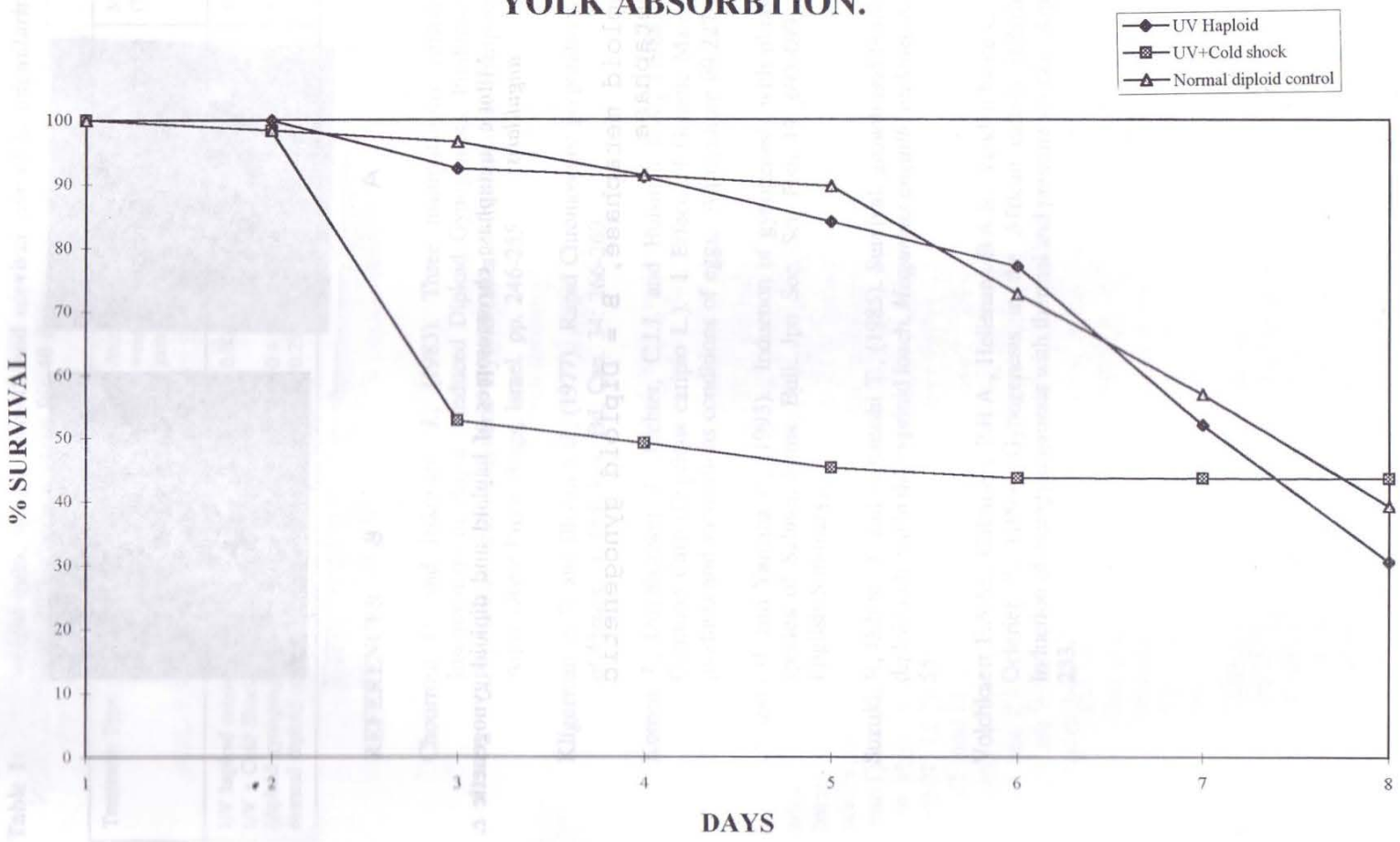
Fig 1 shows the survival of treated and normal larvae of *C. anguillaris* fed with *Moina dubia* in the first eight days after yolk absorption. There was a sharp drop in the number of the UV + Cold shock group (from 98.3% to 52.75) probably as a result of effect of the UV irradiation followed by cold temperature shock. The survival was also stable from day four to day eight in this treatment type. Fig 1 further shows that at day 8, the percentage survival was 30.5% for UV haploid control, 43.6% for diploid gynogens and 39.3% for normal diploid control. This shows that the diploid gynogenesis survived better than either of the two controls.

Plate 1 shows the mitotic chromosomes of haploid (n=27) and diploid gynogenetic (2n=54) *C. anguillaris*. UV control larvae examined were true haploid (Plate 1A). Diploid larvae were obtained in the cold shock group (Plate 1B).

The average weight gain for diploid gynogens was 0.40g in 42 days of feeding trail while the average weight gains for the controls were 0.004g (UV haploid control) and 0.235g (normal diploid control) (Table 1). Moreover, the percentage weight gain in 42 days of feeding trial was 1.45% for UV haploid control, 58.0% for diploid gynogens and 71.2% for normal diploid control. This shows a better response of the normal diploid control to 40% crude protein diet than the diploid gynogens in spite of the initial better growth performance of the diploid gynogenetic fry fed with zooplankton. This strongly sugguest that a different composition of feed be provided for the diploid gynogens in order to maintain its growth superiority. The percentage survival of the fingerlings in 42 of feeding trial is also shown in Table 1. 86.7% fingerlings of diploid gynogens survived. The frequencies of 26.7% and 66.7% survival were recorded for UV haploid and normal diploid control, thus indicating that diploid gynogenetic fingerlings have better survival tendency than either of the controls.

At present, the problem with meiotic gynogenesis as a useful technique for aquaculture practices is the low hatchability which could be explained by the deleterious cold shock effects. There is need to produce highly yielding and surviving fry from mitotic gynogenesis. The application of cold shock at a later time during embryonic development is likely to yield a higher percentage of hatched larvae as a result of the fact that the fertilized eggs at this stage are already water hardened. If this technique is perfected it could enable the production of monosex culture.

FIG 1. SURVIVAL OF TREATED AND NORMAL LARVAE OF *Clarias anguillaris* FED WITH MOINA DUBIA IN THE FIRST 8 DAYS AFTER YOLK ABSORBTION.



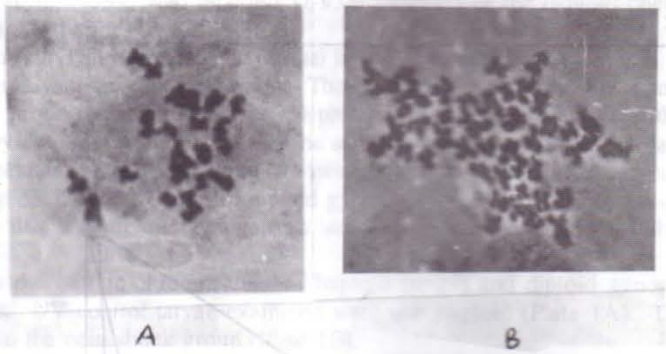


Plate 1: **Mitotic metaphase chromosomes of haploid and diploid gynogenetic *c. anguillaris***

A = Haploid metaphase, B = Diploid gynogenetic metaphase

Table 1: weight gain, specific growth and survival rate of *C. anguillaris* fingerlings fed diet with 40% crude protein for 40 days

Treatment Type	Initial average (%) weight	Final average weight (g)	Average weight gain (g)	Percentage Weight	Duration of Experiment (days)	Mortality (%)	Survival
UV haploid control	0.275	0.279	0.004	1.45	42	11	26.67
UV + Cold Shock (diploid gynogen)	0.690	1.09	0.4	58.0	42	2	86.67
Normal diploid control	0.330	0.565	0.235	71.2	42	5	66.67

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