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Full Length Research Paper

The use of cold shock in inducing triploidy in African mud catfish (*Clarias gariepinus*)

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A study was conducted to induce triploidy in African mud catfish *Clarias gariepinus* using cold shock. The fertilized eggs were exposed at one temperature regime of 0°C with varied shock treatments of 0, 15, 25 and 30 min. Some 3 min after fertilization, the success of triploidy was determined by the presence or absence of bent trunk in the fry as reported by (Aluko et al., 1997). The shock duration of 25 min at 0°C gave the best result of 55% hatch with survival at the end of the exposure regime of 43.5%. Shock exposure of 30 min gave 30% hatch while 15 and 20 min exposure were poor at 15% hatch.

Key words: *Clarias gariepinus*, cold skock regime, triploidy, hatchability.

INTRODUCTION

The development of improved fish seed stocks that can contribute to increased fish production while ensuring protection of biodiversity and the environment is seen as one of the key solutions to meeting the future food demands of the growing world population. Genetics and the successful application of breeding programs in crops and livestock have provided the impetus for the governments in developing countries opportunities to easily and quickly increase food production.

Current interest in polyploidy induction is almost entirely due to its potential application in fish farming, for the pro-duction of triploid and tetraploid fish (Purdom, 1993; Pandian and Koteeswaran, 1998). Induced triploidy is used to produce partially or completely sterile fish, whose 3 chromosome sets impair the meiotic division involved in germ cell formation (Thorgaard and Allen, 1987; Chourrout, 1988; Levanduski, et al., 1990).

The interest in sterility lies in the possibility that this may lead to increased growth. The energy, which would normally be directed for gonadal growth (gonad formation) and reproduction, is thus used for somatic growth (Pandian and Koteeswaran, 1998).

Triploidy induction refers to the production of individuals with three sets of chromosomes and can be induced in fish by inhibiting the second meiotic division followed by the extrusion of the second polar body by shocking eggs shortly after fertilization (Chourrout, 1988; Malison et al., 1993).

The benefit of triploidy to Nigeria aquaculture industry is very enormous. It will help in stemming the incidence of breeding pressures on stock resulting in deformed fish and most importantly it potential association with improved growth and carcass quality for commercial farming purposes.

MATERIALS AND METHODS

Construction of the experimental flow-through system

10 plastic tanks with a volume capacity of 5000 cm^3 each were used along with 3 ½-inch pressure pipes and joints. The tanks were perforated just 8 cm above the tanks base while the pipes were cut into small sizes of 0.30 m and inserted into the hollow holes in the tanks to allow for water drainage (outlet). A long PVC pipe was then joined to the tanks to serve as the main drainage channel. The inlets were constructed and placed above the tanks for gravitational flow..

Selection of broodstock

Care was taken when selecting the male and female brooders. The male brooder selected was large with a slightly swollen reddish urinogenital papilla. While the female brooder had all signs which are attributed to matured female brooders including well rounded, soft projected abdomen, which extended anteriorly beyond the pectoral fins of the genital opening. Also, the genital opening was swollen

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Table 1. Nutrients composition of the fish diet.

Nutrient	Amount
Crude protein	45%
Crude fibre	1.3%
Crude fat	0.4%
Ash	7.3%
Calcium	0.7%
Phosphorus	1.3%
Lysine	4.2%
Methionine	1.3%
Vitamin A	22500 IU(E)/kg
Vitamin D3	2500 IE)/kg
Vitamin E	200 mg/kg
Vitamin C	300 mg/kg
Copper	500 mg/kg
Selenium	0.4 mg/kg

The remnant feed in the culture receptacle was siphoned out to prevent water pollution and subsequent mortality of fry.

and reddish and finally there were good response of free flow of ripe eggs without blood smear, at the application of gentle pressure on the abdomen. The male and female brooders were weighed on a top load measuring scale Camry (Model T1432682).

Administration of ovaprim

Clarias gariepinus female brooder was injected with ovaprim®, at 0.5 ml per Kg of body weight. The injection was done intramuscularly above the lateral line just below the dorsal fin. The injected area was massaged with a finger in order to make sure that the administered ovaprim® dose was evenly distributed throughout the muscle and also to prevent backflow of the hormone. The injected fish was held in a tank for a latency period lasted for 10 h at 24°C overnight water temperature.

Extraction of milt

The milt used for the fertilization process was extracted by sacrificing and dissecting the male, in order to remove the gonad (testis). Before the collection of the milt, the physiological solution was prepared by dissolving 9 g salt (NaCl) / litre of water. The extracted milt was placed into a piece of tissue paper to remove blood and moisture from the milt sac.

Egg stripping

Gentle pressure was applied on the abdomen of the female brooder and ovulated eggs oozed out freely from the genital opening into a clean, dried stainless steel bowl without any contaminant. Spoonful of fertilized eggs were measured for cold shock control experiments.

Fertilization of eggs

The incubation tanks and coldshock medium were already prepared prior to fertilization. The flow-through system was made to run and regulate. Proper aeration was ensured by the use of electric air pumps to which hose and air stones were connected. Aside this, mosquito net was laid in the tanks on which the fertilized eggs were placed for incubation. The milt was poured on the eggs evenly and mixed thoroughly by gently agitating the container.

Post-fertilization cold shock treatment

The cold shock medium was applied by using a mixture of 1 litre of water with 9.5 kg of ice flakes as water bath. 3 min old fertilized eggs were subjected to cold shock at 0°C for a period of 15 min, 20 min, 25 min and 30 min treatments. Mercury in glass thermometer was used to determine the temperature and to ensure the 0°C temperature regime throughout the period of shock treatment. Each beaker in the cold shock medium was removed at its respective time regime (that is, 15, 20, 25 and 30 min) and the eggs were distributed into the various assigned tanks for normal incubation at 24°C, pH 7.44, DO 5 mg/L and salinity 0.0%.

Hatching of fertilized eggs

Hatching is the mechanical and enzymatic process of breaking of the egg shell and release of the larvae. Commencement of hatching was noticed after 23 h in control and 23.5 h of incubation in the treated group. Total hatching was noticed after 32 h of incubation.

Larval rearing

Larval rearing was carried out by placing 300 hatchlings each in replicate plastic tanks. In the first 3 days, the healthy larval were nourished by the yolk deposit and subsequently fed with artemia from the 4th day for 2 weeks.

Fry rearing

1,090 fry each from cold shocked and control group were stocked in the culture receptable (plastic tanks) and fed with artificially prepared 45% crude protein diet *ad libitum* daily for 6 weeks. Sampling for weight was done on a weekly basis.

The remnant feed in the culture receptacle was siphoned out to prevent water fowling and mortality of fry (Table 1).

Growth performance of cold shocked *C. gariepinus* fry (g)

Weekly mean weight of fry was monitored for the duration of the study, the final weight gain ranged from 0.22±0.03 in 20 min treatment to 0.47±0.03 in 30 min of treatment. 30 min cold shock treatment gave the best performance while the control group and 25 min treatment were similar at 0.40 g each. 20 min shock treatment gave the poorest growth of only 0.22±0.03 over the eight week period.

Water quality monitoring

Water quality parameters such as dissolved oxygen, pH, conductivity, salinity and temperature required for growth and other biological processes were monitored weekly. Water in the culture recaptacles was changed daily and aerated with air pumps throughout the period of the study to ensure high water quality and to prevent stress.

Table 2. The hatchibility of *C. gariepinus* larval of cold shock.

Treatment	Hatchibility (%)
Control	95%
Cold shock duration of 15 min	15%
Cold shock duration of 20 min	15%
Cold shock duration of 25 min	55%
Cold shock duration of 30 min	30%

Table 3. Mean (%) weekly survival of *C. gariepinus* fry produced by cold shock.

Week	Control	Coldshock duration			
		15 min	20 min	25 min	30 min
1	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00
2	87.5 ± 5.00	71.5 ± 2.12	77.5 ± 10.61	91.5 ± 0.71	89.5 ± 3.54
3	78.0 ± 9.00	60.5 ± 6.40	71.0 ± 9.90	88.5 ± 0.71	85.5 ± 4.95
4	68.5 ± 0.70	50.5 ± 3.54	54.5 ± 3.54	61.0 ± 1.41	62.0 ± 1.41
5	62.0 ± 0.00	44.0 ± 2.83	43.0 ± 4.24	53.5 ± 3.54	54.5 ± 6.36
6	58.5 ± 0.70	36.5 ± 2.12	36.0 ± 2.83	49.5 ± 3.52	40.0 ± 11.31
7	53.0 ± 1.41	27.5 ± 0.71	31.5 ± 2.12	45.5 ± 3.54	37.5 ± 13.44
8	49.0 ± 1.41	21.5 ± 2.12	30.0 ± 1.41	43.5 ± 4.95	33.5 ± 9.19

Statistical analysis

Data were analyzed graphically and using analysis of variance on SPSS software version 11.

RESULTS AND DISCUSSION

It was observed that the control had the highest hatched fry (95%), while 55% was the highest from the cold shock treated group (Table 2). The objective of this study was to establish applicability of shock treatment to *Clarias gariepinus*, however growth performance of fry also indicate improved weight gain (Table 4) especially at the 30 min shock treatment thus making up for the low survival of treated fish. The mean water parameters measured are presented in Table 5. The mean temperature ranged from 26.5 to 26°C, mean dissolve oxygen from 6.0 to 6.8 mg/L while the mean pH from 7.5 to 7.6 in the coldshock and control groups.

Some abnormal fry were produced by cold shock. The abnormality was a bent trunk. Consequently, they were unable to swim effectively. The abnormal fry died within 14 days.

The present experiment was successful in the induction of triploidy in African mud catfish (*C. gariepinus*), achieving about 55 to 75% (Table 2) triploidy using cold shock 3 min after fertilization at a temperature of 0°C with duration of 25 min. Post fertilization cold shock treatment was applied by using a mixture of ice and water as a waterbath. According to Purdom (1993), it is easy to apply coldshock precisely by using a mixture of ice and water as a waterbath.

It is generally thought that warm water species are more susceptible to cold shock than to heat shock, whereas heat shock is more effective for cold water species (Chourrout, 1980; Nagy 1987). The experiment verified this contention as African mud catfish (*C. gariepinus*) is an inhabitant of warm climate and has been shown to be highly susceptible to cold shock treatments. Cold shock treatment was observed to have a detrimental effect on the fertilized egg of *C. gariepinus*, the hatching and survival in cold shock treated groups were considerably lower than those of control group in this study (Table 3). Such lower hatch and survival rates of triploid individuals compared to control have been reported by other authors (Chrisman, et al., 1983 and Krasznai, et al., 1984).

Similar results have also been reported in other species and have been attributed to factors such as egg quality differences or the susceptibility of eggs from different origins to shock treatments (Johnstone, 1985). Malison et al. (1993), however, have suggested the wide range of ambient water temperature from which brood fish were captured as the probable cause for such variation in their experimental fish, perch (*Perca flavescens*). In the case of *C. gariepinus* such factors in addition to egg quality could have been important. Moreover, the variation in the water temperature (Table 5) during the incubation period under ambient conditions might have also contributed to difference in hatching and survival rates.

An abnormality was found with the fry in the cold treated group and the abnormality observed was a bent trunk. Similar observation has been reported by Aluko et al. (1997) in *C. anguillaris*. The authors suggested that the

Table 4. Mean growth performance of cold shocked *C. gariepinus* fry.

Week	Control	15 min	20 min	25 min	30 min
1	0.05 ± 0.02	0.05 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.04 ± 0.01
2	0.04 ± 0.00	0.05 ± 0.01	0.03 ± 0.02	0.04 ± 0.00	0.04 ± 0.00
3	0.06 ± 0.00	0.09 ± 0.01	0.05 ± 0.02	0.07 ± 0.01	0.06 ± 0.01
4	0.09 ± 0.00	0.11 ± 0.00	0.03 ± 0.03	0.10 ± 0.04	0.09 ± 0.04
5	0.13 ± 0.01	0.16 ± 0.02	0.12 ± 0.04	0.12 0.04	0.15 ± 0.04
6	0.14 ± 0.01	0.16 ± 0.01	0.21 ± 0.12	0.15 ± 0.01	0.21 ± 0.04
7	0.22 ± 0.01	0.31 ± 0.06	0.20 ± 0.03	0.22 ± 0.03	0.39 ± 0.01
8	0.40 ± 0.07	0.45 ± 0.11	0.22 ± 0.03	0.40 ± 0.01	0.47 ± 0.03

Table 5. Mean water quality parameters.

Parameter	Control	15 min	20 min	25 min	30 min
pH	7.58 ± 0.09	7.57 ± 0.08	7.50 ± 0.04	7.49 ± 0.11	7.5 ± 0.08
Salinity (%)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Temperature (°C)	26.5 ± 0.14	26.7 ± 0.09	26.5 ± 0.06	26.6 ± 0.11	26.6 ± 0.05
DO (mg)	6.20 ± 0.07	6.80 ± 0.13	6.20 ± 0.01	6.0 ± 0.05	6.20 ± 0.16
DCO_2 (mg)	0.24 ± 0.01	0.35 ± 0.03	0.18 ± 0.01	0.29 ± 0.11	0.21 ± 0.04

abnormality could be due to chromosome misbehaviour.

In Tables 2 and 3, the survival of the fry posthatch showed that about 55% of fry survived in the cold shock treated hatchlings while about 95% survival was recorded in the control group. This lower early survival in the cold shock group supports the observation (Chourrout, et al., 1986) that triploids have somewhat lower early survivals than their full-sib control. Similarly, Olufeagba and Aluko (1997) reported early low survival in the first few days posthatch in the triploid *Heterobranchus longifilis.* However, there was no significant difference between survivals in the control and other treatments (Table 3) throughout the 8 week rearing period .

The study shows that triploidy can be successfully induced in African mud catfish (*C. gariepinus*) by application of cold shock at 0°C for duration of 25 min. This work can serve as a basis for further work to test and develop standardized shock protocols for a commercial production of triploid *C. gariepinus* in Nigeria. Attempts at producing tetraploid and a hybrid triploid from tetraploid x diploid should be intensified. This way, the adverse effects of shock application might be averted. It is strongly believed that production of genetically sterile African mud catfish (*C. gariepinus*) using the ploidy techniques as in salmonids, tilapia and other fish would be of great benefit to aquaculture in the country by reducing indiscriminate breeding in farms.

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