GYNOGENESIS: AN EFFECTIVE WAY OF CONTROLLING FISH POPULATION AND INCREASING PROFIT IN AQUACULTURE

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ABSTRACT

Gynogenesis is a gene manipulation technology to produce all female fish. Brood stock of Clarias gariepinus were obtained from a fish farm in Mosogar, Delta State, Nigeria. The milt was irradiated with Ultra Violet (UV) light to inactivate the male chromosomes. The fertilized eggs were then cold shocked. One way analysis of variance was use to analyze the data on the haploids, triploids and diploids. Hatchability of fertilized eggs indicated lower hatchability and survival rates in the diploids and the triploids. Analysis of variance showed significant difference in the growth of the diploid and triploid. There were also higher growth rates in the triploid than the diploids.

Keywords: Gynogenesis, triploids, diploids, *Clarias gariepinus.*

INTRODUCTION

In many species of cultured finfish, females exhibit higher growth rates than males and attain larger sizes (Jakupsstovu and Haug, 1988; Bilgin and Celik, 2009) and males mature and slow down growth before reaching a marketable size. Differences in growth rate and timing of sex maturation result in a mixed population of individuals showing considerable variations in sizes and consequently an overall reduction in production. Therefore, there is an increasing interest in generating all female brood stocks for cost efficient aquaculture (Luo et al., 2011).Gynogenesis, sometimes referred to as "pseudogamy", "pseudofertilization" or "parthenogenesis", is a form of unisexual reproduction whereby females produce offspring with all their inheritance from the female parent (solar et al., 1991), although present in nature in several lower phyla (Kiester et al., 1981), lower vertebrates that ordinarily rely on sexual reproduction can resort to facultative parthenogenesis under extenuating circumstances that isolate females from males. Molecular tools have now been applied to the study of unisexual organisms and fascinating insights have emerged regarding the molecular mechanisms that preserve heterozygosity and increase genetic diversity in all-female populations (Neaves and Baumann 2011). Gynogenesis is a genome manipulation facilitating the inheritance of maternal genetic material alone and this technique involves the activation of egg development by genetically inactive spermatozoa and subsequent diploidic restoration (Pandian and Koteeswaran, 1998). Induced gynogenesis is usually achieved by fertilization of eggs with genetically inactivated sperm using radiation or chemical treatments or with heterologous sperm that triggers development without any genetic contribution to the egg (Thorgaard, 1983; Ihssen et al., 1990). Controlled gynogenesis is one of the main methods of genome engineering, which can help to solve problems of fish genetics and selection (Cherfas, 1987; Ihssen et al., 1990).The objective of this study is to determine the performances of triploid and diploid in Cliarias gariepinus using Gynogenesis.

MATERIALS AND METHODS

Study site and sample collection

The experiment was carried out in the biology laboratory, Delta State College of Physical Education, Mosogar, Delta State. Samples used for the research were brood stocks of Clarias gariepinus obtained from a local fish farm in Mosogar, Delta state, Nigeria. The fish were stored in big plastic bowls of clean water to allow for acclimatization. The samples were selected based on both external morphology which included body weights, total lengths, maturity of gonads and agility. The female fish numbering six (6) weighed between 0.8-2kg live weight and the male also numbering three (3) weighed between 0.7 and 1.5 kg.

Gamete collection

Mature female fish were given ova prim injection at the rate of 0.5ml to 1kg body weight of fish and allowed to rest for a latency period of 9hours. After which, the ovulated oocytes were obtained by stripping females and collected into a clean receptacle. The milt were obtained by first killing and dissecting the male fish and extracting the male gonads which was cut and the milt was extracted and diluted with physiological saline solution and refrigerated.

Milt inactivation

Ultra violet (UV) light was used to irradiate part of the collected male gamete. Calibration of the UV lamp (254 nm, VL 115C, 30 W, 220V, 50Hz) was performed by use of a VLX - 3W radiometer. The lamp was warmed up 15 minutes before the onset of the irradiation. The source-filter to sample distance was maintained at 20 cm throughout the experiments, giving an incident dose of 4.000 erg mm-2. Sperm irradiation was performed using 1.5 ml of diluted milt (1:20) placed in a 55 mm diameter Petri dish on ice and continually stirred with a magnet. Irradiated sperm samples were kept refrigerated before use as postulated by Peruzzia and Chatain (2000).

Artificial propagation

Eggs were divided into approximately 3 equal groups (300-500 eggs), held in individual 200 ml beakers and fertilized with 1.5 ml of diluted irradiated or normal sperm as follows. The vials containing the sperm samples were taken from the refrigerator and left at 27°C. The sperm was then added to the eggs and a larger volume of water was added and the eggs left undisturbed. The eggs fertilized with normal milt were to be the control group.

Cold treatments

After 3 minutes of incubation, the eggs fertilized with irradiated milt were divided into two groups: The non shocked group which will produce diploid and the group to be shocked which will produce triploid. Establishment of liable periods for the induction of diploid meiotic gynogenesis by cold shocks was performed by using set values of intensity and duration of treatment and by varying the moment of application (4-7 minutes after). Set values for cold shocks were 1°C for 20 minutes. The non shocked samples were maintained in their beakers at 27°C. Eggs to be cold shocked were transferred into individual plastic vials with perforated mesh and kept in water at 27°C. Cold shocks were applied by soaking the vials in a polystyrene incubator containing ice and water at 1°C. Temperature was constantly monitored throughout the experimentation. Immediately after treatment, eggs were gently rinsed and transferred in 200 ml beakers and incubated with their controls in a thermo regulated incubation system at 27°C.All experiments were replicated four times using egg batches derived from different females.

Feeding and water quality monitoring

Shell free artemia were fed to all the treatments for four weeks. Water quality parameters required for growth such as PH, dissolved oxygen and temperature were monitored and stabilized daily.

Experimental design

Data obtained from the experiment were subjected to one-way Analysis of Variance (ANOVA) and difference between means were separated using Duncan's Multiple Range Tests at 5% confidence interval.

RESULTS AND DISCUSSION

Survival rates of fertilized eggs

There were varying levels of survival rates among the three groups (control, non-shocked group (Diploid) and the post radiation shocked group (Triploid)). The control had between 90-80 % survival rates, the non-shocked group had between 70-40 percent survival rates as the level of radiation increased and the third group had 40 15% survival rates.

Table 1showing the fertilization and survival rates of the different group fertilization

	GROUP 1 Control	GROUP 2 irradiated	GROUP 3 Irradiated + Cold shock
% hatchability	90	70	40%
Survival after 24 hrs	87	60	35
Survival after 48hrs	85	50	20
Survival after 72 hrs	80	40	15

This was also observed in Olufeagba et al., (2001) and Lamadrid-Rose and Boehlert (1988) where they observed a decline in hatchability and survival of fish irradiated with UV light and treated with cold shock. This variation in the water temperature during irradiation and cold shock under ambient conditions might have also contributed to difference in hatching and survival rates

Growth rates

The growth rates obtained for the 4 weeks the fish were cultured indicated higher increase in the mean of 0.21g of the triploids compared to the diploids with a mean of 0.12g and the control with a mean of 0.08g as shown below.

Table 2 showing the growth rates in mean weights of the different groups

	GROUP 1 Control (g)	GROUP 2 Irradiated(g)	GROUP 3 Irradiated + Cold shock(g)
Week 1	0.05	0.05	0.07
Week 2	0.06	0.09	0.14
Week 3	0.09	0.11	0.21
Week 4	0.12	0.24	0.41

This consequent increase in growth has been observed by Frasser et al., (2013), Garner et al., (2008) where triploid did better than diploids and even where less aggressive feeders but had higher growth rates, which is good for aquaculture. Garner et al., (2008) also reported that triploids matured earlier than diploids.

Cold shock

It was observed that some abnormal fry were produced by exposure to cold shock. The resulting abnormality was a bent trunk. Consequently, they were unable to swim effectively and died within 14 days. Similar observation has been reported by Aluko et al., (1997) in C. anguillaris. Aluko et al (1997) suggested that the abnormality could be due to chromosome misbehavior.

Analysis of variance

The result of analysis of variance indicated that there was significant difference ($p<0.05$) in the growth rates of the triploids when compared to the diploids and there was also significant difference ($p<0.05$) between the diploids and control.

CONCLUSION

The importance of gynogenesis in ensuring cheaper fish production through the production of high yielding and fast growing fish cannot be overemphasized. It may be a little expensive but it's worth the while practicing.

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