STUDIES ON THE GENETIC IMPROVEMENT OF THE AFRICAN CATFISH (*CLARIAS GARIEPINUS* BURCHELL, 1822) BY TRIPLOIDY

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ABSTRACT

This study sought to assess the possibility of genetically improving the performance of the African catfish using triploidy techniques. The experiment was carried out using Completely Randomized Design (CRD) with varied temperatures, time spent before shock after fertilisation, and durations of the shock for the triploid production experiment and four treatments replicated thrice for the culture experiment. Fertilised eggs from each female parent stock were subjected to either cold or heat shock treatments. The result showed a significant (p<0.05) increase in the hatching time and survival of the fertilized eggs subjected to different cold and heat shock treatments as compared to the control which recorded the shortest hatching time of 22.48 ± 0.00 hours. The temperature regime of 2°C recorded the highest approximate yield of triploids (98.33 ± 1.66%), while the prolonged duration of the shock of 30 minutes at the temperature regime of 3°C significantly increased (p<0.05) triploid yield. Comparatively, triploids significantly gained more weight, had an increased growth rate, and grew longer than the diploid counterparts with an average of 11.22 ± 1.07 g, 0.013 ± 0.001%, and 21.49 ± 0.47 cm respectively. The highest comparative cost of the diet of 1000 catfish juveniles occurred in triploids with a stocking density of 7 fishes/m² (₦14,132.50). However, the seed, feed, and variable costs consistently decreased with increasing stocking densities across the ploidies. It is therefore concluded that the triploids were superior in both production attributes and cost benefits.

Keywords: Triploids, Catfish, Heat and cool shocks, Cost, Aquaculture

INTRODUCTION

One of the key requirements for attaining sustainability in the practice of aquaculture in recent times is the development and stocking of improved fish seed stocks. Such stock should ensure the protection and preservation of the environment as well as allow for the maintenance of biodiversity (Hammed *et al*., 2009). Genetic improvement programs and the successful application of breeding programs in livestock have provided the option for governments in developing countries to easily and quickly increase food production (Williams *et al*., 2008).

Fish farming in Nigeria is a growing industry with over 2, 658 fish farms as of 2003 (FAO, 2007). Out of these, 2,152 comprise the culture of catfish alone or in polyculture with *Oreochromis niloticus* and *Heterotis niloticus* (Williams *et al*., 2008). The cultured catfish species include *Clarias gariepinus, C. anguillaris, C. Isheriensis, C. submarginatus, Heterobranchus bidorsalis, H. longifilis, Chryscicthyes nigrodigitatus, Bagrus* sp. and *Synodontis* sp. (Williams *et al*., 2008). Among the above-mentioned species, *C.*

gariepinus remains the most cultured species in Nigeria; the second most cultured species in Africa (after *Tilapia*), and the third in the world.

Despite the high potential for the production of *Clarias* species in the country, the industry has not been well established due to some critical limiting factors. One of these limiting factors has been the lack of good quality fish seeds for farmers and producers (Brummett, 2007). This challenge has resulted from the poor performance of available local brood stock and poor management practices in hatcheries as well as the non-existence of any appropriate breeding structure or improved strains in line with global best practices (Ponzoni and Nguyen, 2008).

Other constraints that have resulted in the low productivity of *C. gariepinus* in existing farming systems include difficulties with induced breeding, the low survival rate of larvae and fries, lack of least-cost optimal balanced diets, and poor post-harvest technology. There are also issues of environmental protection, genetic resource conservation, genetic biodiversity, and sustainability of the industry (Ponzoni and Nguyen, 2008).

In a bid to stem the degenerating tides confronting the industry in recent times, there are urgent need to genetically improve the fish stocks. Consequently, several researchers have delved into the problem to find lasting solutions (Aluko *et al*., 1998; Lawson and Ishola, 2010; Olele and Tighiri, 2013). Genetic improvement shortens production time and stress. Although a lot of works have been documented on the attempts to genetically improve fish species through triploids (Benfey and Sutterlin, 1984; Omeji *et al*., 2013; Hammed *et al*., 2009; Lawson and Ishola, 2010), more information is needed on the effectiveness of the genetic improvement methods especially as it relates to the culture of *C. gariepinus* in Nigeria*.* Genetically improved catfish (*C. gariepinus*) would lead to an increase in the nation's fish production. This study aimed at accessing the possibility of improving the genetic performance of the African catfish (*C. gariepinus)* using the triploidy technique. The study assayed both the pre-culture and the culture production parameters The result of this study therefore will serve as a guide on the best methods of genetically improving *C. gariepinus* using triploidy to reduce production time and stress.

MATERIALS AND METHODS

Procurement of Experimental Brood Fish: Nine healthy and mature (1.0 $\pm$ 0.3 kg) mud catfish *Clarias gariepinus* brood stock comprising 6 females and 3 males used in this study were procured from the World Bank-West Africa Agricultural Productivity Programme (WB-WAAPP) Fish Seed Centre within the Faculty of Agriculture, University of Nigeria, Nsukka. The identification of the broodstock and the confirmation of their maturation were carried out according to Ugwu and Mgbenka (2006) and Hammed *et al.* (2009) respectively.

The breeders were separated according to sex and the tanks were labeled accordingly. The separated brood fishes were then fed 5 % of their body weight with a formulated diet containing 40.38% crude protein in divided rations of morning at 8.00 hours and evening at 16.00 hours. All breeder ponds were aerated with an air blower and the physicochemical parameters of the water were constantly monitored. The acclimation of the brood fish lasted for two weeks.

Experimental Design: The study consists of the pre-culture and the culture experiments. The experiment adopted the Completely Randomized Block Design (CRBD), comprising 12 treatments and control (treatments varied along temperature in °C): Time spent before shocking the eggs after fertilization: durations of the shock); A (Control), B (1:03:15), C (1:03:30), D (1:04:15), E (1:04:30), F (2:03:15), G (2:03:30), H (2:04:15), I (2:04:30), J (3:03:15), K (3:03:30), L (3:04:15), M (3:04:30). The temperature range for cold shock were 1: 1°C, 2: 2°C, 3: 3°C, while the heat shock temperature range was 1: 36°C, 2: 38°C and 3: 40°C. All treatments were replicated thrice for both cold and heat shocks of the pre-culture experiment.

The culture experiment comprised eight treatments with three replicates each. The culture treatment was based on the stocking density; triploid (7/m^2, 14/m^2, 21/m^2 and, 28/m^2)

and diploid ($7/m^2$, $14/m^2$, $21/m^2$ and $28/m^2$) respectively.

Administration of Gonadotrophic Hormone: A total of 0.5 and 0.25 ml/kg of the commercial fish ovulation hormone (ovaprim) was injected intra-muscularly into the dorsal muscles above the lateral line of the female and male fish respectively, just below the anterior part of the dorsal fin, using a graduated syringe (2 ml) at 22.00 hours. The pond water temperature was 28^0C. The needle was placed parallel to the fish, pointing posteriorly at an angle of approximately 30^0. After the injection, the injected area was rubbed with one finger to distribute the hormone suspension evenly throughout the muscles (Delince *et al*., 1987).

Procurement of sexual products: Stripping of the female breeders took place 8 hours after injection at a pond water temperature of 28^0C (Delince *et al.,* 1987). Milt was obtained from the male breeders by sacrificing the male and dissecting the testis, and the squeezed-out milt was rinsed with drops of 0.7% physiological salt solution.

Fertilization: The fertilization of the sexual products was done according to the methods of de Graaf and Janssen (1996). Female breeders that were injected 8.00 hours earlier were removed from the well-labelled ponds and stripped into dry bowls. The already dissected and removed testes were lacerated to release the milt. The released milt was allowed into a petri dish and 25 ml of normal saline was mixed with the milt. The normal saline mixed milt was then used to fertilize the eggs by gently mixing the stripped eggs with the sperm using well-sterilized poultry feathers.

Triploidy Induction: Fertilized eggs from each of the female parent stock were subjected to either cold or warm treatments.

Cold treatments: Fertilized eggs were coldly shocked using ice flakes as a water bath, 3 minutes and 4 minutes after fertilization at respective temperatures of 1, 2, 3, and 4^0C for a period of 15- and 30 minutes duration each. Model H-9269 digital thermometer was used to determine the temperature and to ensure the eggs were shocked at the appropriate temperature regime throughout the exposure period. Successfully shocked eggs in the cold shock medium were removed at their respective temperature-time regime and the eggs were evenly distributed on the incubation tray for incubation. Water quality parameters within the incubation period were within the range for the fish species (Hammed *et al*., 2009).

Heat temperature treatments: This was carried out by subjecting the fertilized eggs to either of the temperature regimes of 36, 38, and 40^0C for a duration of 1 and 2 minutes using a hot oven, and the successfully warm shocked eggs, were placed in incubators at ambient temperatures. The third batch of fertilized eggs was allowed to proceed with normal incubation at ambient temperature without any temperature treatment or alteration. This served as the control for the experiment. Incubation chambers for treatment groups were continuously aerated and on a continuous flow-through system. Other incubation and nursing processes were carried out according to the methods of de Graaf and Janssen (1996).

Pre-culture Production Parameters: The production parameters were evaluated following standard procedures. These include hatching time, hatchability (Olele and Tighiri, 2013), survival to first feeding, and approximate yield of triploids (Hussain and McAndrew, 1994; Olaniyi and Omitogun, 2014).

Culture Production Parameters: Juvenile triploid and diploid catfish (*Clarias gariepinus)* were each stocked in 1 m^2 plastic culture tanks at their respective stocking densities of 7, 14, 21, and 28 fishes/m^2 (Ogugua *et al*., 2011). The stocking density of 7, 14, 21, and 28 for the triploids represents treatments; A, B, C, and D respectively, while the same stocking density for the diploids represents treatments; E, F, G, and H. The stocked juveniles were acclimated for 2 weeks to the takeoff of the culture trial. The culture trial lasted for 20 weeks during which the fish were fed 5 % of their body weight with a

standard formulated diet containing 40.38% protein (Table 1). The water in the tanks was refreshed regularly and the physicochemical parameters of the water were maintained within the acceptable limits for the culture of catfish. The culture production parameters evaluated include weight gain, specific growth rate, and total length (Chiu, 1989).

Table 1: Ingredients and proximate compositions of diet feed to the experimental ploidy catfishes

Ingredient	Composition (%)
Maize	20.00
Soya bean meal	20.00
Fishmeal	32.00
Palm kernel cake	10.00
Ground nut cake	10.00
Carboxymethyl cellulose	12.00
Vitamin-mineral Premix*	0.50
Vitamin C	0.20
Salt (NaCl)	0.30
Proximate composition	
Dry matter	90.30
Crude protein	40.38
Ether extract	5.53
Crude fibre	3.10
Ash	5.20
NFE	47.81
Gross energy (Kcal/kg)	4649.70

** Contains: thiamine (B1) 2.5 mg, riboflavin (B2) 2.5 mg, pyridoxine 2.0 mg, pantothenic acid 5.0 mg, inositol 3 mg, folic acid 0.75 mg, para-amino benzoic 2.5 mg, choline 200 mg, niacin 10.0 mg, cyanocobalamin (B12) 10.0 mg, menadione (k) 2.0 mg, $CaHPO_4$ 727.8 mg, $mgSO_4$ 1275 mg, KCL 60 mg, $FeSO_4$ 50.0 mg, $ZnSO_4$ 250 mg, Mn_2SO_4 5.5 mg, $CuSO_4$ 2.5 mg, $CoSO_4$ 0.79 mg, $CaClO_3$ 0.48 mg, $CrCl_3$ 0.3 mg*

Cost Benefit Analysis

Estimate of the comparative cost of diet: The estimate of the cost of feeding 1000 triploid and diploid catfishes reared in plastic culture tanks for 20 weeks with a standard diet was estimated using their fortnightly feeding levels and weight gains (Eyo, 2003).

Seed cost: The cost contribution of seed to the production cost of the triploid and diploid catfishes grown at different stocking densities for 20 weeks was computed using the procedure outlined by Lipton and Harrel (2004).

Feed cost: The cost contribution of feed to the production cost per pound (0.7kg) of fish was also computed using the procedure outlined by Lipton and Harrel (2004).

$C_{variable}$: The estimate of major variable costs for producing a pound (0.7kg) of fish is computed with the formula: $C_{variable} = C_{seed} + C_{feed}$ (Lipton and Harrel, 2004). Where: C_{seed} = Cost contribution of seed for producing a pound (0.7 kg) of fish and C_{feed} = Cost contribution of feed to produce a pound (0.7 kg) of fish.

Water Quality Parameters: The water quality parameters of each of the tanks were monitored bi-weekly and 70 % of the water was replaced with fresh water every week while the entire water was completely changed every fourth night. The physicochemical parameters monitored include; temperature using model H-9269 Multi thermometer at a depth of 25 cm, dissolved oxygen (DO) ascertained using a DO meter (Infitek model P10), pH ascertained using a digital pH meter, and ammonia (NH_3) monitored using the methods of APHA (1989).

Data Analysis: Data resulting from the pre-culture experiment was analyzed using the one-way analysis of variance, while data from the culture experiment was subjected to multivariate analysis of variance using the SPSS version 23 (IBM, 2015), and the differences between significant means were separated using the Least Significant Difference Test at $p<0.05$.

RESULTS AND DISCUSSION

Pre-culture Production Parameters

Hatching time: There was a significant ($p<0.05$) increase in the hatching time of the fertilized eggs subjected to different cold and heat shock treatments as compared to the control which recorded the shortest hatching time of 22:48 ± 0.00 hours. However, treatments G, I, J, K, and M showed significantly ($p<0.05$) longer hatching time (Table 2). Heat shock treatments' hatching time did not seem to have followed any definite pattern with the cold shock.

Table 2: Mean preculture production parameters of catfish (*Clarias gariepinus)* eggs subjected to cold and heat shock treatments after fertilization

Treatment	Hatching time (HAF)		Hatching rate (%)		Survival to first feeding (%)		Approximate yield of triploid (%)	
	Cold Shock	Heat Shock	Cold Shock	Heat Shock	Cold Shock	Heat Shock	Cold Shock	Heat Shock
A (Control)	22.48 ± 0.02^{b}	22.48 ± 0.02^{a}	91.00 ± 1.00^{f}	91.00 ± 1.00^{f}	76.67 ± 1.20^{g}	76.67 ± 1.20^{d}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
B (1:03:15)	23.08 ± 0.02^{c}	23.03 ± 0.03b^{b}	27.33 ± 1.20^{d}	55.00 ± 1.1^{e*}	44.00 ± 2.08^{e}	51.67 ± 1.67^{bc}	93.33 ± 1.67^{d}	83.33 ± 1.67^{def}
C (1:03:30)	0.00 ± 0.00^{a}	23.30 ± 0.30^{b*}	0.00 ± 0.00^{a}	50.00 ± 1.15^{d*}	0.00 ± 0.00^{a}	50.67 ± 0.67^{bc}	0.00 ± 0.00^{a}	86.67 ± 1.67^{ef}
D (1:04:15)	23.12 ± 0.02^{c}	23.03 ± 0.03^{b}	26.67 ± 1.67^{d}	54.33 ± 0.67^{e*}	45.67 ± 0.67^{ef}	53.33 ± 2.03^{bc}	96.67 ± 1.67^{b}	80.00 ± 2.89^{d}
E (1:04:30)	0.00 ± 0.00^{a}	23.07 ± 0.03^{b*}	0.00 ± 0.00^{a}	49.33 ± 0.67^{d*}	0.00 ± 0.00^{a}	52.00 ± 0.58^{bc}	0.00 ± 0.00^{a}	88.33 ± 1.67^{f}
F (2:03:15)	23.12 ± 0.03^{c}	23.03 ± 0.03^{b}	30.67 ± 0.33^{e}	48.00 ± 0.58^{d*}	45.67 ± 2.33^{ef}	59.67 ± 9.67^{c}	95.00 ± 2.89^{c}	78.33 ± 1.67^{d}
G (2:03:30)	23.19 ± 0.01^{d}	23.08 ± 0.04^{b}	9.67 ± 0.88^{b}	44.67 ± 0.88^{c*}	11.67 ± 1.67^{b}	48.67 ± 0.88^{b}	98.33 ± 1.67^{d}	81.67 ± 1.67^{de}
H (2:04:15)	23.09 ± 0.01^{c}	23.08 ± 0.04^{b}	27.33 ± 1.33^{d}	48.33 ± 0.33^{d}	45.67 ± 1.45^{bc}	49.33 ± 0.67^{b}	93.33 ± 1.67^{b}	83.33 ± 1.67^{def}
I (2:04:30)	23.18 ± 0.04^{d}	23.10 ± 0.03^{b}	9.33 ± 0.67^{b}	42.00 ± 1.15^{c}	17.67 ± 1.20^{bc}	49.67 ± 1.20^{b}	98.33 ± 1.67^{c}	86.67 ± 1.67^{ef}
J (3:03:15)	23.11 ± 0.02^{c}	23.18 ± 0.02^{b}	30.87 ± 0.94^{e}	17.67 ± 1.45^{b}	50.33 ± 0.88^{f}	10.67 ± 1.45^{a}	85.00 ± 2.89^{b}	72.67 ± 1.45^{c}
K (3:03:30)	23.22 ± 0.02^{d}	23.18 ± 0.02^{b}	15.75 ± 0.92^{c}	11.00 ± 1.00^{a}	13.33 ± 1.67^{cd}	7.67 ± 0.88^{a}	91.6 ± 1.67^{c}	68.33 ± 1.67^{c}
L (3:04:15)	23.11 ± 0.02^{c}	23.15 ± 0.03^{b}	28.25 ± 5.12^{d}	18.00 ± 1.15^{b}	39.75 ± 10.02^{f}	9.00 ± 0.58^{a}	81.67 ± 1.67^{b}	61.6 ± 1.67^{b}
M (3:04:30)	23.22 ± 0.01^{d}	23.23 ± 0.02^{b}	14.00 ± 1.00^{c}	11.33 ± 1.33^{a}	12.50 ± 2.50^{d}	7.00 ± 0.58^{a}	91.67 ± 1.67^{c}	58.33 ± 1.67^{b}

* = significantly different means at p<0.05 using t-test between the heat treatment, ^{abcd} = Mean values with different letter superscripts on a column for each variable differ significantly at p<0.05

There was no significant (p<0.05) difference in the hatching time of all the heat-shocked treatments except the control (Table 2). The prolonged hatching time may be attributed to the postponing effects of shock on embryonic development (Hammed *et al.,* 2009). The deleterious effect of shock on developing embryos was observed in prolonged duration of cold shock (30 minutes) which caused total mortality (0.00 hours hatching time) of the fertilized eggs of the different treatments in the temperature regime.

Hatchability/hatching rate: The hatching rate among the cold and heat-shocked treatments decreased significantly (p<0.05) as compared to the controls. While the heat shock had a higher hatching rate at a lower waiting time after fertilization before shock application (Treatment B – H), cold shock had a higher hatching rate at a higher waiting time (Treatment J – M). The temperature regime of 3˚C for cold shock and 36°C for heat shock gave the best hatching rate among the treatments. The variation in cold shock effect may be attributed to factors such as egg quality differences or the susceptibility of eggs from different origins to shock treatments (Shelton *et al.*, 1986; Gheyas *et al.*, 2001; Hammed *et al.*, 2009). However, the damaging effect of the heat shock on the developing embryo could have resulted from the differences in the egg size and quality; cell size, and the DNA content of the nucleus of the respective cell (Marx and Sukumaran, 2007).

Therefore, very low or high temperatures affect the hatching rate (Table 2).

Survival to first feeding: For cold shock, increased duration of shock application significantly reduced ($p<0.05$) the survival to first feeding of the hatched eggs. The control treatment had the highest number of hatchlings that survived till their first feeding followed by treatment J (3:03:15). In a similar way, for heat shock, they were a significant reduction in survival of the hatchlings from the control. However, among the treatments, the best survival to first feeding among the heat-shocked treatments occurred in the temperature regime of 38^0C (Table 2). The reduced number of hatchlings that survived to first feeding after treatment may be due to the effects of the ploidy syndrome on the larvae (Parven and Gallardo, 2014). Also, the reduced survival to the first feeding of the hatched eggs with increased duration of shock application could result from undue disturbances of important stepwise processes of the fertilized eggs by the application of shock (da Silva *et al*., 2007). From the study, the time waited after fertilization (TAF) before the application of shock did not affect the survival to first feeding. This suggested that the TAF used in the study (1 – 3 minutes) may be within the tolerable time limit for the fish species, while the sub-optimal temperature regime of 40°C may be outside the acceptable temperature range for the induction of triploid in *C. gariepinus* (Parven and Gallardo, 2014).

Approximate yield of triploids: Approximate yield of triploids increased with an increase in the time waited after fertilization before the application of heat (TAF). The temperature regime of 2^oC recorded the highest approximate yield of triploids (I – 98.33 ± 1.66%), while the prolonged duration of the shock of 30 minutes at the temperature regime of 3^0C significantly increased ($p<0.05$) triploid yield. More so, the highest yield of triploids among the heat-shocked treatments occurred in treatment E (36:04:02), followed by treatment I (38:04:02,) and C (36:03:02). Prolonged duration of shock significantly increased ($p<0.05$) the approximate yield of triploids. Also, prolonged TAF (time waited after fertilization before the application of shock) of 4 minutes had a higher triploid yield than the yield of 3 minutes. The temperature regime of 40°C recorded the least triploid yield among all the treatments except the control (Table 2).

There exists a strong relationship between the shock temperature regime, duration of shock, and the time waited after fertilization (TAF) in the optimization of triploid yield. Thus, the correct combination of these factors is necessary to maximize triploid yield (Dillion, 1988). An effective combination of temperature regime, time after fertilization, and duration of shock for optimal triploid induction without a serious decrease in hatchability and survival rates must be determined by trial and error (Arai, 2001). Even though such protocols have currently been developed for a few species (Pandian and Koteeswaran, 1998; Arai, 2001; Okunsebor *et al.,* 2015; Meng *et al.,* 2023), this study showed significant ($p<0.05$) differences in the approximate yield of triploids among the cold and heat-shocked treatments. Triploid yield increased with an increase in the duration of shock (30 minutes) in some temperature regimes. However, the increased duration of shock affected the hatching rate and survival rates of the larva. This deleterious effect has been attributed to the effects of shock regime and timing on polar body expulsion, and damage of the formed spindle threads of the egg during cell fission (Dillion, 1988; Marx and Sukumaran, 2007; Parven and Gallardo, 2014).

Culture Production Parameters

Several intrinsic and extrinsic factors largely affect the growth and well-being of every organism including fish. In the case of *C. gariepinus*, several authors have shown that growth is intrinsically and extrinsically affected by the gene, health status, stocking density, water quality, feed, and feeding regime (Hossain *et al*., 1998; Hammed *et al*., 2009; Olele and Tighiri, 2013).

Weight gain: The effects of ploidy level and stocking density on the weight gain of *C. gariepinus* juveniles indicated that on average,

triploids significantly ($p<0.05$) gained more weight than the diploids (Table 3). There were also significant differences ($p<0.05$) among *C. gariepinus* juveniles stocked at different densities. The results showed that the weight gained was dependent on the stocking density with lower stocking density gaining significantly ($p<0.05$) more weight across both the triploids and diploids. The observed higher mean weight gain of the triploids over the diploids in the experiment may be attributed to the extra set of chromosomes associated with triploids (Hammed *et al*., 2009; Olele and Tighiri, 2013; Achegbulu *et al*., 2013). Several other researchers have also reported that the extra set of chromosomes associated with the triploids may be connected with the sterility of triploids which allows them to divert more energy for growth instead of sexual gonadal development (Hammed *et al*., 2009; Agbebi *et al*., 2010; Achegbulu *et al*., 2013). The observed significant differences in the weight gain at the different stocking densities with the triploids stocked at lower densities outperforming their counterparts stocked at higher densities as well as outperforming the diploids of similar densities suggests the superiority of the triploids in terms of culture requirements and growth performance and that triploids require lower stocking rates and less stress full culture conditions than the diploids and as such should be treated as a new species with specific culture requirements. The studies on the weight gain of triploids have serious implications for the physiochemistry and the aquaculture economics of catfish in the tropics.

Specific growth rate: There were significant differences ($p<0.05$) in the specific growth rates (SGR) of the triploid and diploid *C. gariepinus* juveniles reared separately at varied stocking densities in plastic culture tanks (Table 3). Also, there were significant differences ($p<0.05$) in the SGR within the triploids stocked at varied stocking densities. The SGR within the diploids reared at different stocking densities was not significantly different ($p<0.05$). The higher specific growth rate observed with the triploids suggested the possession of a more intrinsic capacity for growth when given optimal conditions. This was what played out with the lower stocking density of the triploids having better conditions for growth. Thus, it may be proper to suggest that triploids be given better culture conditions different from the ones currently used for the diploids.

Total length: From physical observations and morphometric measurements, the triploids were significantly longer than their diploid counterparts (Table 3). Total lengths within ploidies, however, were not affected by stocking densities. The observations of this study on the effects of stocking density on the total lengths of the catfishes were in line with the reports of Anibeze (2000) on a related species *H. longifilis.* However, the observation did not follow the same trend as reported by Greenberg and Dahl (1998) on brown trout *Salmo trutta* L. reared in a mixed cobble-bottomed habitat, which probably may be a result of the differences in species.

Cost Benefit Analysis

Estimate of the comparative cost of diet: The comparative cost estimate of feeding 1000 diploid and triploid catfishes reared in plastic culture tanks at different ploidy levels and stocking densities for 20 weeks with standard diets revealed cost values corresponding to the weight gains and diet requirements. There were differences in the overall comparative cost of diets of the different ploidies and stocking densities at the end of the 20-week culture trial. The highest comparative cost of the diet of 1000 catfish juveniles occurred in triploids with TSD 7 (₦14, 132.50). This was followed by the triploid's stocking density of 14 (₦ 10, 867.35) and DSD 14 (₦ 9, 949.00) (Table 4). Thus, comparative diet costs generally increase with increasing duration of culture. The triploids had higher diet cost per unit weight gain in all the treatments (stocking densities). Diet cost per unit weight gain also generally increased with increased duration of culture trial. The study observed that the triploids had higher diet costs than the diploids. This agreed with Achegbulu *et al.* (2013) who reported that the triploids may not have optimally converted their feeds despite their having significantly different weight gains.

Table 3: Mean culture production parameters of *Clarias gariepinus* juveniles grown separately in plastic culture tanks at different ploidy levels and different stocking densities

Treatments (Stocking densities)	Weight gain (g)		Specific Growth rate (%)		Total lengths (cm)	
	Triploid	Diploid	Triploid	Diploid	Triploid	Diploid
7	18.50 ± 3.08^{c*}	9.61 ± 1.78^{b}	0.017 ± 0.001^{c*}	0.013 ± 0.001^{a}	22.88 ± 1.27^{a*}	19.26 ± 0.98^{a}
14	12.05 ± 1.85^{b*}	9.21 ± 1.54^{b}	0.014 ± 0.001^{b}	0.013 ± 0.001^{a}	21.35 ± 0.94^{a*}	19.55 ± 0.85^{a}
21	8.18 ± 1.12^{ab*}	6.09 ± 0.81^{a}	0.011 ± 0.001^{ab}	0.011 ± 0.001^{a}	20.79 ± 0.74^{a*}	19.47 ± 0.66^{a}
28	6.16 ± 0.60^{a}	5.81 ± 0.75^{a}	0.010 ± 0.000^{a}	0.011 ± 0.001^{a}	20.94 ± 0.66^{a*}	19.86 ± 0.64^{a}
Overall	11.22 ± 1.07*	7.68 ± 0.67	0.013 ± 0.001*	0.012 ± 0.000	21.49 ± 0.47*	19.54 ± 0.39

* = significantly different means at p<0.05 using t-test between the ploidy, ^{abcd} = Mean values with different letter superscripts on a column for each variable differ significantly at p<0.05

Previous studies (Benfey, 1999; Koedprang and Na-Nakorn, 2000) had reported that before sexual maturity there will be an increase in feeding rate with a less optimal feed conversion among triploids. Therefore, there are possibilities of reducing the cost of the triploids before sexual maturity.

Seed cost: The cost contribution of seed (C_{seed}) to the overall production cost of the fish grown in plastic culture tanks at different stocking densities for 20 weeks revealed a significant difference ($p<0.05$) between the seed cost of the triploids and the diploids when compared between ploidies (Table 5). However, the stocking densities did not affect the seed cost significantly ($p<0.05$) but the triploids with a stocking density of 7 recorded the highest cost (₦ 22.35 ± 1.18). Seed cost among the triploids consistently decreased with increasing stocking density but followed no definite order among the diploids. When the specific stocking density between the diploids and the triploids where compared, it was observed that the seed cost of the triploids was consistently higher than that of the diploids. This finding corroborates the reports of Nwichi and Adogbeji (2013) and Chatchaiphan *et al*. (2016) who attributed the variations observed to a relatively higher mortality rate of triploid seeds resulting from the effects of triploidization. Triploid seeds are usually less hardy with relatively lower survival rates than their diploid counterparts resulting in increased cost of labour and material requirements for production. Lipton and Harrel (2004) opined that the overall cost of seed for any aquaculture remains a function of the purchase/production price of the seed as well as the survival rate.

Feed cost: The triploids had significantly higher ($p<0.05$) feed cost (₦136.78 ± 25.05) than their diploid counterparts (₦ 78.95 ± 8.61). There were also significant differences ($p<0.05$) in the feed costs of the different stocking densities within each ploidy with triploid stocking density of 7 (₦ 265.92 ± 4.50) having the highest cost (Table 5). However, the feed costs consistently decreased with increasing stocking densities in the ploidies. The significantly higher feed cost of the triploids relative to the diploids could have possibly resulted from the higher mean weights, mean weight gains, and mortality rates of the triploids compared to the diploids. Also, the decreasing feed costs per fish of the ploidies with increasing stocking densities suggests that triploid and diploid catfishes require more feed, gain more weight, and generally perform optimally at low stocking densities. Achegbulu *et al.* (2013) reported that the high feed consumption rate of triploid catfish resulted in a higher cost of production thereby leading to lower profit when compared to the diploid. Polyploidy tends to increase feeding but not proportionate with growth rate before sexual maturity which consequently raises the cost of feeding and lowers profit, especially at the adult age (Fast, 1998; Koedprang and Na-Nakorn, 2000; Schafhauser-Smith and Benfey, 2001).

Table 4: Summary of the comparative cost of feeding 1000 triploids and diploids *Clarias gariepinus* juveniles with standard diets at varying stocking densities and ploidy levels for 20 weeks

Ploidy level	Stocking Density	Parameter	Weeks											Total
			0	2	4	6	8	10	12	14	16	18	20	
Diploid	**DSD 7**	CW (g)	1000 × 24.90	1000 × 26.90	1000 × 29.02	1000 × 33.00	1000 × 38.27	1000 × 44.41	1000 × 51.51	1000 × 59.96	1000 × 74.90	1000 × 84.90	1000 × 121.00	1000 × 588.77
		DR (g)	1245	1345	1451	1650	1913.5	2220.5	2575.5	2998	3745	4245	6050	29,438.50
		CD (₦)	373.5	403.5	435.3	495	574.05	666.15	772.65	899.4	1123.5	1273.5	1815	8831.55
		GR (g)	-	2	2.12	3.98	5.27	6.14	7.1	8.45	11.94	10	36.1	93.1
		DC/W (N/g)	-	0.6	0.64	1.2	1.58	1.84	2.13	2.54	3.58	3	10.83	27.93
	DSD 14	CW (g)	1000 × 25.40	1000 ×27.55	1000 × 30.02	1000 × 34.32	1000 × 39.82	1000 × 45.82	1000 × 52.67	1000 ×66.47	1000 × 77.47	1000 × 93.47	1000 × 117.47	1000 × 663.3
		DR (g)	1270	1377.5	1501	1716	1991	2291	2633.5	3323.5	3873.5	4693.5	5873.5	33165
		CD (₦)	381	413.25	450.3	514.8	597.3	687.3	790.05	997.05	1162.05	1402.05	1762.05	9949.5
		GR (g)	-	2.15	2.47	4.3	5.5	6	6.85	13.8	11	16	24	92.07
		DC/W (N/g)	-	0.65	0.71	1.29	1.65	1.8	2.1	4.14	3.3	4.8	7.2	27.62
	DSD 21	CW (g)	1000 × 24.85	1000 × 27.03	1000 × 29.33	1000 × 32.78	1000 × 35.00	1000 × 40.00	1000 × 45.59	1000 × 55.69	1000 × 66.39	1000 × 76.79	1000 × 85.79	1000 × 574.83
		DR (g)	1242.5	1351.5	1466.5	1639	1750	2000	2279.5	2784.5	3319.5	3839.5	4289.85	28741
		CD (₦)	372.75	405.45	439.95	491.7	525	600	683.85	835.35	995.85	1151.85	1286.85	8622.3
		GR (g)	-	2.18	2.3	3.45	2.02	5	5.59	10.1	10.7	10.4	9	60.94
		DC/W (N/g)	-	1	0.69	1.1	1	1.5	2	3.03	3.21	3.12	2.7	19.35
	DSD 28	CW (g)	1000 × 25.00	1000 × 27.03	1000 × 30.10	1000 × 33.15	1000 × 35.15	1000 × 40.10	1000 × 45.59	1000 × 55.69	1000 × 66.19	1000 × 75.10	1000 × 83.10	1000 × 561.79
		DR (g)	1250	1351.5	1505	1657.5	1757.5	2005	2279.5	2784.5	3309.5	3755	4155	280895
		CD (₦)	375	405.45	451.5	499.25	527.25	601.5	683.85	835.35	992.85	1126.5	1246.5	8426.85
		GR (g)	-	2.03	3.07	3.05	2	4.95	5.49	10.1	10.5	8.91	8	58.1
		DC/W (N/g)	-	0.61	0.92	0.92	0.6	1.5	1.65	3.03	3.15	2.67	2.4	17.43
Triploid	**TSD 7**	CW (g)	1000 × 26.40	1000 × 30.35	1000 × 35.82	1000 × 43.04	1000 × 52.07	1000 × 64.10	1000 × 76.53	1000 × 103.40	1000 × 131.74	1000 × 167.80	1000 × 211.40	1000 × 942.65
		DR (g)	1320	1517.5	1791	2152	2603.5	3205	3826.5	5170	6587	8390	10570	47132.5
		CD (₦)	396	455.25	537.3	645.6	781.05	961.5	1147.95	1551	1976.1	2517	3171	14139.75
		GR (g)	-	3.95	5.47	7.22	9.03	12.03	12.43	26.87	28.34	36.06	43.6	197.43
		DC/W (₦/g)	-	1.2	1.64	2.2	2.71	3.61	3.73	8.06	8.5	10.82	13.08	55.55
	TSD 14	CW (g)	1000 × 25.44	1000 × 32.15	1000 × 35.24	1000 × 39.72	1000 × 45.72	1000 × 52.85	1000 × 61.07	1000 × 78.81	1000 × 97.36	1000 × 120.01	1000 × 136.12	1000 × 724.49

		DR (g)	1272	1607.5	1762	1986	2286	2643.5	3053.5	3940.5	4868	6000.5	6806	36224.5
		CD (₦)	381.6	482.25	528.6	595.8	685.8	792.75	915.9	1182.15	1460.4	1800.06	2041.8	10867.35
		GR (g)	-	6.71	3.09	4.48	6	7.13	8.22	17.74	18.55	22.65	16.11	110.68
		DC/W (₦/g)	-	2.01	1	1.34	1.8	2.14	2.5	5.32	6	7	4.83	33.21
	TSD 21	CW (g)	1000 × 26.00	1000 × 29.14	1000 × 32.67	1000 × 36.70	1000 × 41.96	1000 × 47.76	1000 × 53.57	1000 × 64.78	1000 × 77.78	1000 × 93.08	1000 × 109.35	1000 × 612.79
		DR (g)	1300	1457	1633.5	1835	2098	2388	2678.5	3239	3889	4654	5467.5	30639.5
		CD (₦)	390	437.1	490.05	550.5	629.4	716.4	803.55	971.7	1166.7	1396.2	1640.25	9191.85
		GR (g)	-	3.14	3.52	4.03	5.26	5.8	5.81	11.21	13	15.3	16.27	83.35
		DC/W (₦/g)	-	1	1.1	1.21	1.6	1.74	1.74	3.36	3.9	4.59	4.88	25.01
	TSD 28	CW (g)	1000 × 26.46	1000 × 29.99	1000 × 34.89	1000 × 38.12	1000 × 43.02	1000 × 48.29	1000 × 52.90	1000 × 58.94	1000 × 67.37	1000 × 77.08	1000 × 88.33	1000 × 565.39
		DR (g)	1323	1499.5	1744.5	1906	2151	2414.5	2645	2947	3368.5	3854	4416.5	28269.5
		CD (₦)	396.9	449.85	523.35	371.8	645.3	724.35	793.5	884.1	1010.55	1156.2	1324.95	8480.85
		GR (g)	-	3.53	4.9	3.23	4.13	5.27	4.61	6.04	8.43	9.71	11.25	61.1
		DC/W (₦/g)	-	1.1	1.47	1	1.24	1.58	1.4	1.81	2.53	2.91	3.4	18.33

Key: *DSD = diploid stocking density, TSD = triploid stocking density, CW = comparative weight, DR = diet required, CD = cost of diet, GR = growth rate, DC/W = diet cost/weight*

Table 5: Seed, feed and variable costs of triploid and diploid catfish (*Clarias gariepinus*) juveniles grown separately in plastic culture tanks at varied stocking densities for 20 weeks

Stocking density	**Seed cost**		**Feed cost**		**Variable cost**	
	Triploid	**Diploid**	**Triploid**	**Diploid**	**Triploid**	**Diploid**
7	$22.35 \pm 1.18^{b*}$	13.33 ± 0.00	$265.92 \pm 4.5^{d*}$	112.61 ± 3.2^{d}	$288.28 \pm 3.53^{d*}$	125.94 ± 3.2^{d}
14	$21.68 \pm 1.02^{ab*}$	13.72 ± 0.39	$151.02 \pm 0.41^{c*}$	101.53 ± 0.5^{c}	$172.3 \pm 61.1^{c*}$	114.98 ± 0.42^{c}
21	$21.36 \pm 0.31^{ab*}$	13.38 ± 0.05	$79.31 \pm 1.04^{b*}$	52.29 ± 1.99^{b}	$101.34 \pm 1.04^{b*}$	65.71 ± 1.99^{b}
28	$20.92 \pm 0.3^{a*}$	13.33 ± 0.00	50.88 ± 0.22^{a}	49.37 ± 0.96^{a}	$71.80 \pm 0.45^{a*}$	62.70 ± 0.96^{a}
Total	$21.58 \pm 0.38^{*}$	13.44 ± 0.01	$136.78 \pm 25.05^{*}$	78.95 ± 8.61	$158.45 \pm 25.16^{*}$	92.34 ± 8.61

* = significantly different means at $p<0.05$ using t-test between the ploidy, ^{abcd} = Mean values with different letter superscripts on a column for each variable differ significantly at $p<0.05$

Variable cost: The variable cost contribution to the final production of the triploids and diploids showed that the variable cost of the triploids was significantly higher ($p<0.05$) than that of diploids both at the ploidy levels and their corresponding stocking densities. There was an observed downward trend in the variable costs of the fish with increasing stocking densities. In all the stocking densities, the triploids consistently had higher variable costs than the diploids. This result agrees with the report of Achegbelu *et al*. (2013) and may be attributed to the relatively higher cost of the variable cost components (seed cost and feed cost) of the triploids when compared with the diploids.

Water Chemistry: The water chemistry parameters (pH, temperature, DO and ammonia) values observed during the 20-week study in all the treatments did not show significant varying patterns among the ploidy levels except for ammonia. The triploids showed significantly reduced ammonia concentrations with stoking densities of 14 and 28 (Table 6). Ammonia affects water quality in several ways. In fishes, ammonia induces homeostasis and toxicity through shared mechanisms. High concentrations of ammonia can lower the dissolved oxygen levels in water, making it more difficult for aquatic organisms to survive. Additionally, ammonia can react with other chemicals in water to form harmful compounds like nitrite and nitrate (Edwards *et al*., 2023).

Relationship between Water and Production Parameters: In triploids, it was observed that DO showed significant positive correlations with condition factor and Specific growth rate while ammonia negatively correlated with specific growth rate (Table 7). In diploids, pH was positively correlated with specific growth rate and weight gain while DO was positively correlated with condition factor (Table 8). The DO concentration of an aquatic environment reflects its prevailing physical and biological processes (Jain *et al.,* 2022). Its regular supply is required for the optimal functioning of all aquatic organisms except for anaerobic bacteria. The required quantity of DO should, however, be maintained through scientific management of the pond to get high yields of the most important environmental parameters exerting tremendous influence on the growth and production in aquatic ecosystems through its effects on feed consumption and metabolism as well as other environmental processes (Bulbul Ali and Mishra, 2022). The pH of a typical aquaculture system has a significant influence on the toxicity several other physico-chemicals have on fish. In our study, pH had a positive correlation with weight gain and corroborates with Akongyuure and Alhassan (2021) which may be attributed to the complex interactions between the stocking density's biomass, physiological processes and the hydrogen ions.

Conclusion: The produced triploids were superior in both production attributes and cost benefits. Therefore, the utilization of triploids by fish farmers would help in increasing productivity and as well the profitability of the venture. In the production process, the study suggests that the time after fertilization before applying shock used in the study (1 – 3 minutes) may be within the tolerable time limit for the fish species while the sub-optimal temperature regime of 40°C may be outside the acceptable temperature range for the induction of triploid in *C. gariepinus.* The stocking density of 7 fishes/m^2 is more effective in improving the production traits of the produced triploids.

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Table 6: Mean water chemistry parameters of triploid and diploid *Clarias gariepinus* juveniles reared differently at varying population densities for 20 weeks in plastic culture tanks

Water parameters	Ploidy level	Treatment (Stocking densities)			
		7	14	21	28
pH	Triploid	6.95 ± 0.08	6.87 ± 0.058	7.07 ± 0.10	7.12 ± 0.09
	Diploid	7.01 ± 0.09	6.93 ± 0.05	7.05 ± 0.10	7.08 ± 0.10
	T- value	-1.83	-1.290	0.244	0.431
Temperature	Triploid	26.75 ± 0.13	26.74 ± 0.15	26.77 ± 0.12	26.74 ± 0.12
	Diploid	26.78 ±0.12	26.74 ± 0.11	26.76 ± 0.12	26.75 ± 0.12
	T- value	-0.717	0.000	0.256	-0.206
Dissolved oxygen	Triploid	5.02 ± 0.09	4.53 ± 0.12	4.22 ± 0.10	4.09 ± 0.10
	Diploid	5.10 ± 0.13	4.44 ± 0.11	4.15 ± 1.99	4.07 ± 0.08
	T- value	-0.905	1.337	-0.978	0.357
Ammonia	Triploid	0.01 ± 0.00	0.020 ± 0.00	0.02 ± 0.00	0.03 ± 0.00
	Diploid	0.01 ± 0.00	0.026 ± 0.00	0.02 ± 0.00	0.09 ±0.02
	T- value	1.294	3.151**	0.092	-2.872**

*** = significantly different means at p<0.01 using t-test between the ploidy*

Table 7: Correlation matrix showing the relationship between water parameters and triploid production parameters

Production parameters	Condition factor	Specific growth rate	Weight gain	pH	Temperature	Dissolved oxygen	Ammonia
Condition factor	1						
Specific growth rate	0.369**	1					
Weight gain	0.386**	0.758**	1				
pH	0.071	0.01	0.112	1			
Temperature	0.043	0.157	0.133	0.006	1		
Dissolved oxygen	0.570**	0.217*	0.04	-0.176	-0.021	1	
Ammonia	-0.074	-0.226*	-0.28	0.016	-0.044	-0.002	1

*** Correlation is highly significant at p<0.01, * Correlation is significant at p<0.05*

Table 8: Correlation matrix showing the relationship between water parameters and diploid production parameters

Production parameters	Condition factor	Specific growth rate	Weight gain	pH	Temperature	Dissolved oxygen	Ammonia
Condition factor	1						
Specific growth rate	0.201*	1					
Weight gain	0.259*	0.736**	1				
pH	0.043	0.306**	0.332**	1			
Temperature	0.090	0.036	0.095	0.174	1		
Dissolved oxygen	0.567**	0.085	0.226*	-0.238**	0.052	1	
Ammonia	-0.082	-0.047	-0.032	0.027	0.060	-0.018	1

*** Correlation is highly significant at p<0.01, * Correlation is significant at p<0.05*

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