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The Production of Triploid *Clariobranchus* in Indoor Hatchery

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Abstract: *A study was carried out to determine the effect of cold shock on hybrid Clariobranchus on the process of producing triploid. One Heterobranchus bidorsalis male broodstock of weight 2kg and age 36 months was selected to cross with one Clarias gariepinus female broodstock of weight 1.5kg and age 12-15months. The female was injected above the lateral line with hormone (ovaprim) at rate of 0.5ml/kg after a latency period of 12 hours.Fish with eggs freely oozing out on slight touch were stripped into a dry bowl, the eggs were fertilized and divided into two parts in duplicates (A1, A2, B1, B2) and incubated. Triploidy was induced by subjecting the fertilized female eggs to a temperature of 5^{o}c for 3(three) to 4(four) minutes. A total of 100 fries was stock in each water rearing receptacle and initial weight of diploid is (0.0019±0.001) stocked in B1 and B2 and triploid treatments (0.0019±0.001) stocked in A1 and A2, fries was counted to monitor their daily survival and growth rate on weekly basis for eight weeks. The final weights of diploid were (0.756±0.161) and triploid (1.62±0.080) and their percentage of survival rate were 48% for triploid and 70% for diploid. The change in length for triploid (3n) was from 0.68cm to 5.12cm and the diploid fish from 0.54cm to 5.00cm.The growth rate was monitored and analysis of variance (ANOVA) was used to record the change in weight and length of fish. The triploid strain performs better in growth than diploid strain.*
Keywords*: Triploid, Diploid, Strain, Broodstock and hormone*

I. Introduction

Food is a necessity in life cannot be ignored, It's essence in life can only be second to air and water. There are basically two major components of food which could either be food crop component and animal protein component. Animal protein source on the other hand can be further divided into fish, poultry and livestock. The clarid constitutes an excellent food fish of high commercial value. Infact, the catfish species are very important to the sustainability of aquaculture industry in Nigeria (Owodeinde and Ndinele, 2011). The feeding habit of Nigerians tends to support this assumption and this trend started manifesting from the late 1980s.

Hybridization is the production of progeny of parents from different lines, strains and species. It is one of the genetic improvements in aquaculture industry which has been recognized as a tool for stock improvement and management purposes. Several studies have demonstrated that *Clarias gariepinus and Heterobranchus bidorsalis* hybrid exhibit superior growth, improved survival and general hardiness than true breed of either *Clarias gariepinus* or *Heterobranchus bidorsalis* (Madu *et al* 1992, Madu and Aluko, 1999, Aluko, 1996, Dada and Olarewaju, 1996). Most of these studies have focused on stock manipulations and growth performance at different dietary compositions in indoor and outdoor concrete tanks (Madu *et al,* 1993, Aluko, 1995). However, there is lack of adequate scientific information on the growth performance of hybrid catfish (*HeteroClarias*) under semi-intensive pond condition. Artificial propagation of fishes is a requisite for Chromosome manipulation. Control of spawning can be by habitation manipulation or through direct hormonal intervention.
The main rationales for the use of these techniques in fish culture are the production of inbred lines and the production of monosex or sterile populations (Colombo *et al*, 1995).Gynogenesis and triploidy, in particular, have been induced with variable success in several fresh water species for which artificial fertilization techniques is developed.

In the case of *Androgenesis* and *gynogenesis,* either the female or male DNA, respectively, must be neutralized before the eggs are activated, while in the case of ploidy manipulation both parental genomes are left intact. Chromosome manipulation involves two basic treatments after obtaining fresh gametes (Thorgaard and Allen, 1986). For androgenesis, the female gamete is inactivated by (uv) irradiation. Treated eggs are activated by normal spermatozoa, and then diploidized by shocking to interrupt the first mitotic karyokinesis. In order to prevent chromosome separation, the shock is timed to coincide with metaphase and must be sufficiently severe to disrupt microtubule and spindle fiber formation. Selection of the type of shock (thermal-cold or hot- or pressure) depends on effectiveness and ease of application and must be optimally combined into a protocol for maximum yield of progeny. Further, because the rate of development is inversely desperation dependent, either the pre-shock incubation temperature must be standardized or the shock time must be calibrated to the temperation effect. Absolute shock time (minutes post-activation) can be transformed with reference to an index of development rate (Colombo et al 1995). Triploids are not viable as their diploids strains, because of their low rate of survival in the early stage. This has been attributed to the shock applied on to them to prevent extrusion

of the second polar body, producing aquatic organism with desired traits. The desire goal is to produce offspring that performs better than both parental species. Among the culturable fin fish in Nigeria catfish is the most sought after fish species, very popular with fish farmers and consumers. It commands very good commercial value in Nigeria market. The catfish is very important to the sustainability of the aquaculture industry in Nigeria. The blending of high survival rate and fast growth rate into the hybrid of *Heterobranchus bidorsalis* and *Clarias gariepinus* is a voracious omnivore feeding on a wide range of food from live animal prey through aquatic plants to plankton organism (Madu *et al* 1999). Studies on the hybridization of catfish families abound, but in bowls rearing medium is limited. These studies report the production, growth of hybrid (male *Heterobranchus bidorsalis* and female *Clarias gariepinus*) in a confined environment.

II. Materials And Methods

DESCRIPTION OF THE STUDY AREA

The experiment was carried out at the Teaching and research farm of Delta State University Abraka, Asaba campus. From the months of March to May 2013.

SELECTION OF BROOD STOCK

The broodstock were selection from the Delta State University Broodstock tank and care was taken when selecting the male and female brooders.

THE FEMALE SELECTION WAS BASED ON THE FOLLOWING

- Swollen and soft abdomen
- Reddish or pinkish urinogenital organ
- Release of eggs on slight pressure to the stomach
- The edges of the stomach were uneven

One female fish weight 1.5kg and age 12-15 month was selected from the broodstock tank.

THE MALE BROODSTOCK WAS SELECTED BASED ON THE FOLLOWING

- Aggressiveness of the the male fish {Heterobranchus bidorsalis}
- Reddish reproductive Organ
- Extruching papilla that touches the base of the pectoral fin.

One male fish weight 2kg and age 36 months was selected

COLLECTION OF THE BROODSTOCK

After taking note of the criteria needed in selecting broodstock, One (1) female *Clarias gariepinus* and one (1) male *Heterobranchus bidorsalis* were collected for the experiment to commence.

Hypophysation (Administration of Hormone)

The broodstocks collected from the broodstock tank were disinfected by dipping them into water (10 liters) containing 30g of common salt for (2) minutes. *Clarias gariepinus* female fish and *Heterobranchus bidorsalis* male fish were injected with hormone (ovaprime) at the rate of 0.5ml per kilogram body weight of the fish. The injection was done intramuscularly above the lateral line just below the dorsal fin at an angle of 45^0, for the female while the males was also injected at the rate of 0.1ml hormone (ovaprime). The injected area was massaged with a finger in order to make sure that the administered hormone (ovaprime) dose was evenly distributed throughout the muscle and also to prevent backflow of the hormone. The injected broodstocks were returned to solitary confinement for a latency period of 9 hours at room 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 9g salt (Nacl)/liter of water. The extracted milt was washed in saline solution to remove blood stain. The milt was extracted from the sac by making some cut on the milt sac.

Egg stripping

The egg was stripped at a time when the eggs were freely oozing out of the fish on slight tough after the latency period. 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; care was taken to prevent blood and water from coming in contact with the stripped eggs.

Fertilization/incubation

For fertilization to take place the stripped eggs were mixed with the milt from the milt sac by making some incision on the sac for the milt to come out. The milt was mixed with the eggs inside the bowl with the use of saline solution, and mixed thoroughly for fertilization to take place effectively.
The fertilized eggs was divided into 2 (two) A and B and incubated for 3 (three) minute.

Cold shocking

The part A of the fertilized eggs was retrieved and cold shocked for a period of 3-5 minute at a temperature of 5^0c while the B part was allowed to go through the normal process of incubation. Cold shocking was carried out by retrieving the kaka ban holding the part A eggs from the incubator and placing it in a bowl containing ice using a monitoring thermometer to observe the temperature. An aerator was used to keep the temperature constant.

Hatching and larva rearing

Hatching is the mechanical and enzymatical process of breaking the eggs shell and the release of the larvae. Commencement of hatching was noticed within 23-28 hours of incubation depending on the temperature. The hatched fries were fed after three days of rearing with capsulated artemia for two weeks and commercial extruded feed (coppens) of sizes 0.3 mm – 1.5 mm for four weeks and feeding was carried out at 08:00 hrs and 20:00 hrs. dead fries and unhatched eggs were removed on daily basis to avoid contamination of the water.The weight and length of the fishes were taking using sensitive scale 0.1g and meter rule of 0.1 cm.

Water quality management

Water quality parameters such as dissolved oxygen, pH, salinity. Temperature and other physico chemical requirements were monitored and stabilized.

Data analysis

Data collected and computed from the experiment were analyzed with one way ANOVA using Duncan Multiple Range Test (DMRT) to separate the mean at 5% level of significance.

III. Results And Discussion

Table 1 showing the changes in mean weight (g)

week	Triploid	Diploid
1	0.001920a± 0.001	0.001940a± 0.001
2	0.014400a ± 0.001	0.014400a±0.002
3	0.066000a± 0.005	0.51720a±0.003
4	0.096800a± 0.007	0.84440a± 0.010
5	0.247000b±0.018	0.150200a±0.023
6	0.575200c+0.039	0.404000b± 0.014
7	0.751000d±0.034	0.612000c±0.043
8	1.620000e±0.080	0.756600c±0.161

Mean with the superscript on the same column are not significantly different {p<0.05} where, ±= standard error of the mean, wg =weight Gain LG= length gain, SGR= Specific growth rate and SR = survival rate.

Table 2 showing the change in length (cm)

week	Triploid	Diploid
1	0.6800a± 0.037	0.5400a± 0.024
2	1.3480b±0.313	0.9220b±0.030
3	2.4600c±0.136	2.0200c±0.073
4	2.4600c±0.128	2.1000c±0.114
5	3.2600d±0.150	2.7600d±0.050
6	4.0800e±0.101	3.5400e±0.087
7	4.6000f±0.109	3.9800f±0.124
8	5.1200g±0.193	5.0000g±0.044

Mean with the same superscript on the same column are not significantly different {p<0.05} where ± standard error of the mean, WG= weight gain, LG=length gain, SGR=specific growth rate and SR= survival rate.

Treatment	initial weight	Final weight	Specific growth rate	Survival rate	Mean weight
Treatment (3n)	0.001920	1.620000	5.2247317	48	1.61808
Treatment (2n)	0.001940	0.756600	4.626901084	70	0.75466

Treatment	Initial length	Final length	Specific growth rate	Survival rate	Mean length
Treatment(3n)	0.6800	5.1200	5.2247317	48	4.44
Treatment(2n)	0.5400	5.0000	4.626901084	70	4.46

In table 4.1 week one at {p<0.05}.there is no relationship between the triploid length {$0.54^{a}\pm0.024$}. But in week two at the same significant level {p<0.05} there is a relationship between triploid {$1.34^{b}\pm0.313$} and diploid {$0.9220^{b}\pm0.030$} in week three four, five, six, seven and eight shows no relationship between triploid and diploid.

Triploidy induction is the possession of more than the normal two sets of chromosomes per cell. The result of this experiment revealed the use of cold shock to produce triploid in hybrid *(Clarias gariepinus* and *Heterobranchus bidorsalis).* This involves the application of shocks to eggs shortly after fertilization to cause the retention of the second polar body. A set of chromosome normally lost when meiosis 11 is resumed (Tave, 1993). The research work involves the use of broodstock whose eggs were cold shocked. Hatching was observed at 22 and 28 hours of incubation for diploid and triploid (hybrid) About 4% of the fries hatched has it trunk bent a situation observed and reported by Aluko *et al* (1997) in Clarias angullaris and taken as an indication of production of triploid. the abnormality could be due to the chromosome misbehaviors. It is worth mentioning that the percentage abnormality observed here was low compared with what was reported by Manickam (1991) for *Clarias batrachus* when he observed 13.5% abnormality. The result from table 1shows that the cold shock which is treatment A (triploid) had the highest weight gain with mean value 1.62g, compared to treatment B (diploid) with mean value 0.75g. Fish growth and consequently increase in biomass is of major interest to the fish culturist, the fish nutritionist and the fishery biologist. Priede and Secombes (1991) explained that growth of farmed fish is best described in terms of weight rather than length, since the ultimate product is usually sold in terms of weight.

Table 1 shows the comparism between the growth performances of fingerlings produced by cold shock and control.

The result in table 1 shows that the cold shock treatment (A) has the highest length gain of mean value 5.12cm compared to the diploid treatment (B) of mean value 5.00cm. The significance difference that existed amongst all samples was (p>0.05).The result also shows that there was increase in specific growth rate of the triploid treatment which is treatment(A) which has the mean value 5.22% while the diploid treatment (B) has the specific growth rate of mean value of 4.62%. The survival rate in table 1 showed that the diploid treatment (B) has the highest value of 70% and triploid 48%. A short fall of about 42% in the triploid tank might be from the shock and handling process ontogeny in nature. The cold shock alone will result in high mortality. Olufeagba and Aluko (1997) reported early low survival in the first few days after hatching in triploid *Heterobranchus longifilis*. The experiment verified this contention as African mud catfish *(Clarias gariepinus)* is an inhabitant of warm climate and has been shown to be highly susceptible to cold shock treatments. The cold shock treatment was observed to have detrimental effect on the fertilized eggs of *Clariobranchus*. The hatching and survival rate in cold shock treated groups were considerably lower than that of the diploid as reported by Chrisman *et al* (1983).The result shows that cold shock fish has lower rate of survival compared to diploid.*Clarias gariepinus* being a tropical fish and can readily respond to exposure to low temperature for a considerable time. Nwachi (2011) reported that triploid grows faster and bigger compared to diploid fish.We can opine that production of triploid can solve food security issues in Nigeria because of its dual purpose uses which is for interploid, that is the use of tetraploid female to cross with diploid male to produce triploid which would eliminate the need to continually create triploid manually, a process that leads to low survival rate at rearing to maturity.Triploid grows faster than diploid both in weight and length, and therefore attract higher price in the market.Triploid hatchability was low due to the cold shock treatment on their eggs. After the initial, low survival rate of triploid at the early stage, survival rate stabilized and improved after ten days of the hatchery. Percentage of both diploid and triploid were equal during the experiment stage in experimental tank.

Tave (1992) discovered that a triploid which has undergone hardness of shocking effect and survive, can withstand environmental and health hazard. Tave (1992) was of the opinion that triploid usually utilize the energy that is made for gamete development to fasten growth and also, they are larger than diploid because of their ability to convert feed more efficiently than diploid.The shocking effect can stabilize the survived triploids, thus making percentage survival of both diploid (2n) and triploid (3n) equal (100%). Similar success in triploid induction has been reported in channel catfish (Wolter *et al*. 1982). Common carp (Gervai *et al*. 1980).

IV. Conclusion/Recommendtion

From the experiment, triploid strain performs better in growth than diploids strain, and so therefore should be recommended for aquaculture for more profit. Based on findings of the present study, we can recommend that cold shock should be used in production of triploid *Clarias gariepinus.*

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