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# HATCHING AND GROWTH OF TRIPLOID AND TETRAPLOID OF (*Heterobranchus longifilis*) AND (*Hetrobranchus bidorsalis*) SUBJECTED TO ERYTHROCYTES MEASUREMENT

***[1]Hassan, Y. Kabir, [1]Kabiru, A. Nuhu, [1]Farida, I. Mashi, Abdullahi, M. Yahaya, [2]Oateju, O. Philip, [2]Mohammad, S. Bununu, [2]Mohammed, Ismail, Garba, A. Abubakar, [3]Labaran, H. Bashir, [4]Abubakar, Y. Galambi, [5]Bala, M. Sani**

[1]Department of Biological Sciences,Faculty of Life Sciences,Federal University,Dutsinma,Katsina State.
[2]Department of Fisheries and Aquaculture,Faculty of Agriculture,Bayero University,Kano, PMB 3011,Kano State,Nigeria.
[3] Department of Biological Sciences,Rabiu Musa Kwankwaso College of Advanced and Remedial Studies,Tudun Wada,Kano State.
[4]Department of Chemistry,Faculty of Physical Sciences,Federal University,Dutsinma,K atsina State.
[5]Department of Statistics,Joseph Sarwuan Tarka University,Makurdi,Benue State.

*Corresponding authors' email: kabirhassanyahaya@gmail.com

## ABSTRACT

The study experimented (to exposed the hatching and growth) of triploid and tetraploid of heterobranchus longifilis and heterobranchus bidorsalis subjected to erythrocyte (measured),The purposed was to produce better stock of heterobranchus species in terms of growth performance through erythrocyte measurement,also to determine diploid, triploid,and tetraploid red blood cells for aquaculture benefits.This application is one of the major areas of biotechnology whose application to fish breeding lead to increase fish yield. triploid induction is a process whereby an entire chromosome set is added to a fertilized egg. Normal individuals are diploid (2N), receiving one chromosome set (1N) from each parent. Triploid (3N) individuals have an additional chromosome set derived from the second polar body (1N). The second polar body is produced during an egg's meiotic cell division and is normally lost soon after fertilization. If appropriate shock is applied soon after fertilization, the second polar body is retained by the fertilized egg, thus producing a triploid individual. The fish was separated into three tanks of 500 litres water holding capacity of 1m x $1m^3$ representing the treatments. The data on red blood cells analysis and erythrocyte measurement were analysed using duncam multiple wide range test.This study shows that triploidy and tetraploidy could be induced in (H.longifilis and H.bidorsalis) by cold shock treatment, 3 minutes after fertilization at $5^0$C for 20 minutes while tetraploidy could be induced by administering cold shock treatment, 30 minutes after fertilization at $5^0$C for 10 minutes. This study has also shown that erythrocyte measurement can be used to ascertain ploidy level in heterobranchus species.It was concluded from the erythrocyte measurement that the red blood cells (RBC) in tetraploid fish were larger than red blood cell of both triploid and diploid fish.

**Keywords**: Hatching, Growth, Triploid, Tetraploid (H. longifilis, H. bidorsalis)

## INTRODUCTION

Triploid and tetraploid (production,this) is a term that describes the presence of three or four or more homologous sets of chromosomes in a fish. Particularly triploidy are the most common forms of polyploidy. They refer to those fish containing three sets of homologous chromosomes and tetraploid contains four. In some invertebrates and vertebrates, including reptiles, amphibians and teleosts, triploid can be induced in the laboratory resulting in viable individuals.The inhibition of the second meotic division of the egg, shortly after fertilization, is effectively achieved by shock treatments of the eggs (Thorgard, 1983; Ihssen, Mokay, Macmillan, and Philips 1990). These (treatment) are based on pressure, thermal, chemical or cold shocks that cause destabilization of microtubules thus affecting centrosomes that are needed to form the mitotic spindle (Komen and Thorgard, 2007). Consequently, cell division of the egg is interrupted, thus inducing the production of a triploid fish (3n) that has two sets of chromosomes of maternal origin (2n) and a set of chromosomes of paternal origin (1n). Finding adequate combination of type of shock, duration and timing after fertilization prior to application is crucial for each fish species (Ihssen *et al*., 1990; Felip *et al*. 2001a). As triploid fish exhibit an extra amount of chromosomes, germ cells cannot correctly undergo meiotic division.Therefore, these fish are usually genetically sterile animals showing significant alterations that affect their gonadal development,but tetraploid has two pairs that can reproduce one day. In general, triploid females exhibit a reduced gonado-somatic index with ovaries showing oogonia and primary oocytes, without any endocrine signs of maturation. On the other hand, triploid males show a similar gonado-somatic index to that of diploid with impaired spermatogenesis affecting late meiosis and thus blocking spermiogenesis. Although the profile in males is similar to that of diploid, triploid males rarely produce sperm. Nevertheless, the spermatozoa from triploid males, if produced, are aneuploid and thus, their fertilization ability is limited (Benfey, 1999).Polyploidy is one of the major areas of biotechnology whose application to fish breeding lead to increase fish yield. Many fishes are known to be relatively tolerant to chromosomal manipulation especially in the early stages of their development and this gives the advantage of easy application of polyploidy to their breeding procedure, in order to improve on their (seed). However, the use of genetic manipulations especially polyploidy to improve aquaculture productions has been of use in practical management programmes in developing countries (Food and Agricultural Organization, 2010)(Fast and Young 2020) reported that triploid induction is a process whereby an entire chromosome set is added to a fertilized egg. (Normal individuals are diploid (2N), receiving one chromosome set (1N) from each parent. Triploid (3N) individuals have an additional chromosome set derived from the second polar body (1N). The second polar body is produced during an egg's meiotic cell division and is normally lost soon after fertilization. If appropriate shock is applied soon after fertilization, the second polar body is retained by the fertilized egg thus producing a triploid individual while ,tetraploid received additional two chromo somes).Triploid has been induced by culturists and researchers in large numbers of fish, shellfish and other animals that have external fertilization. Reasons for inducing triploid usually relate to either sterility (triploids are functionally sterile), improved growth performance, or some other improved triploid quality. Because triploids are sterile, they can be released to the wild without danger of becoming established (exotic introductions) or of contaminating local gene pools where the species in question is established. This is an important consideration where cultured species have been altered through genetic selection. Always, triploids grow faster compared with diploids. There is also evidence that with some species, triploids are less aggressive.Triploid induction combined with sex reversal is also a useful technique for developing all male or all female cultivars (Feist *et al.* 1996). Because triploid is a method to produce sterile fish, it has been considered as a potential solution for the rearing improvement of fish farming species.(According to) Hulata, (2001) reported that due to this sterile condition, growth performances in triploid may result better than those of diploid (Ihssen *et al*, 1990; Felip *et al*., 2001a). Although polyploid technology is applied in practical aquaculture involving many other species of commercial interest (Hulata, 2001). Additionally, triploidization may be considered as an alternation for the use of genetically modified organisms as well as for mitigating the genetic impact of escapees from farmed fish on wild populations. Protocols for the induction of triploidy and tetraploid have been described for several species of interest in aquaculture. They include several families of freshwater species (*Clarias* species) among others (Ihssen *et al*, 1990; Felip *etal*., 2001a; Piferrer *et al*., 2007).According to Karlmarx and Sanjeeviraj, (2005) chromosome engineering has immediate application in fish farming, because it improves strains of catfish for better growth, assures disease resistance, allows for higher fecundity and increase their tolerance to environmental conditions. Triploids have been created in fishes using thermal shocks (Dunham *et al*., 2003). (Koedprang, and Na-Nakron,2000), recorded high hatchability of 72.5% in the production of triploid *Silver carp* when cold shock was applied for a duration of 10 minutes while, Hammed *et al*., (2010) recorded 55% hatchability in triploid induced eggs using cold shock method for a duration of 25 minutes at $0^0$C. Giuliano and Envoy (2006) stated that exposure for five minutes post fertilization was enough to induce 100% triploidy in *Rhamdia quelen* fish.Lawson and Ishola (2010) reported that cold shock treatment showed better growth rate and lower survival rate compared to diploid *Clarias gariepinus*. Normala *et al*., (2010) reported that triploid catfish had lower hatchability rate and survival rate, but higher growth rate compared to diploid catfish. Gheyas *et al*., (2001), reported that induced triploidy in newly fertilized eggs of *H. fossilis* using cold shock duration for 10 minutes at $20^0$C applied 3 minutes after fertilization was the best.Gima(2009) reported that triploid *Clarias gariepinus* grew better than diploid flathead catfish although the diploids were more aggressive than the triploids. Herbst (2002) stated from a study on induction of tetraploidy in Zebrafish (*Danio rerio*)and the Nile Tilapia (*Oreochromis niloticus*)that both fish species gave good growth performance when crossed with diploid Zebrafish (*Danio rerio*)and Nile Tilapia (*Oreochromis niloticus*). It has produced triploid Zebrafish (*Danio rerio*)and Nile Tilapia (*Oreochromis niloticus*) respectively.Venkatachalam, Venkatachalam, Ganesh, and Aathi (2012), reported from a study on induction of triploidy Catfish through cold and heat shock in *Clarias batrachus* that the eggs were exposed to different temperatures for induction of triploidy. Maximum yield of hatchlings (54.6%) were obtained after the administration of heat shock treatment for 2 minutes after fertilization. Maximum number of triploids (3n=75) 89% were obtained after the temperature treatment of 38°C for one minute. In a cold shock treatment of eggs at 2°C for 15 minutes, the maximum yield of 68% were obtained. Several studies confirm the sterility of triploid fish. One-year-old triploid male and female common carp had gonads that were undeveloped and were sterile (Cherfas *et al*., 1994). Experimentally, there have been extremely rare occasions where triploid males produced small numbers of viable progeny (Dunham, 2003).Female and male triploid wall eye and yellow perch were found to have retarded gonadal development, thus leaving individuals reproductively inactive. Mud loach, *Misgurnus mizolepis* was reported to have suppressed gonadal development when triploidized. Functional sperm from triploid males resulted in aneuploidy individuals that were unviable. Long-term sterility was demonstrated in white bass and hybrid striped bass, which had reduced and dysfunctional gonads at 5 years of age Kerby, Everson, Harrell, Griger, Starling, Revels. (2002). Testosterone levels were found to be similar for triploid European sea bass males (Felip *et al*., 2000a). The effectiveness of ploidy induction for triploid and tetraploid rainbow trout was found to be affected by components (Blanc, Chourrout, and Krieg, 1987). Triploid female *silver carp* did not undergo vitellogenesis (Koedprang and Na- Nakorn, 2000), and female triploid European sea bass had lower hepatosomatic indices, possibly indicating a lack of oestradiol-mediated hepatic synthesis of vitellogenin (Felip *et al*., 2001).

**Objective of the Study**

Determine hatching and growth of triploid and tetraploid *of (heterobranchus longifilis and bidorsalis)* for fisheries advancements.

## MATERIALS AND METHODS

**Study Area**

The study was conducted in the farm of the Department of Fisheries and Aquaculture, College of Forestry and Fisheries,University of Agriculture ,Makurdi,Benue State.

***Selection of Broodstock***

The three male and three female broodstock of (*heterbranchus longifilis and heterobranchus bidorsalis) was* used for the study.

***Ovaprim Hormone Injection***

*H.longifilis and H.bidorsalis* female brooders (was injected with ovaprim, at 0.5 ml per Kg of body weight).The injected fish were held in a tank for a period of 11 hours overnight water temperature at $26^0$C.

***Collection of Milt***

The milt used for the fertilization was extracted by dissecting male fish to obtain the gonads (testis). Prior to the collection of the milt, physiological saline solution was prepared by dissolving 9 g salt (NaCl) / litre of water. (The extracted milt was placed into a piece of tissue paper) to drain blood and moisture from the milt sac.

**Egg Stripping**
Gentle pressure were applied on the abdomen of the female brooder in order to allow the ovulated eggs to ooze out freely from the genital opening into a clean, dry stainless steel bowl without contaminants. A spoonful of fertilized eggs was measured for cold shock experiments.

***Fertilization of Eggs***
The incubation tanks and cold shock medium were previously prepared prior to fertilization. Proper aeration was ensured by the use of electric air pumps to which hose and air stones were connected. Aside this, mosquito netting was laid in the tanks on which the fertilized eggs were placed for incubation to help hatchability. The milt was evenly poured on the eggs and content of the tanks mixed thoroughly by gently agitating the containers.

***Fertilization of Post Cold Shock***
The fertilized eggs were separated into three (tank) of 500 litres water holding capacity of 1m x $1m^3$ representing the treatments A, B and C . Treatment A was diploid, treatment B, triploid and treatment C, tetraploid. 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. Three (3) minutes old fertilized eggs were subjected to cold shock for treatments B, (B1 and B2) at $5^0$C temperature for 20 minutes while treatment C, (C1 and C2) of thirty (30) minutes old fertilized eggs were subjected to cold shock for about $5^0$C (temperature for about) ten (10) minutes. Mercury in glass thermometer was used to determine the temperature. The cold medium was maintained at $5^0$C temperature throughout the period of cold shock treatment for both treatments B and C respectively. Each beaker used for the cold shock medium was removed at its respective time regime (that is, 10 and 20 minutes) 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% (Hammed *et al*. 2010).

***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 observed after 23 hours incubation in the (control) and 23.5 hours in the treated groups. Total hatching was observed after 32 hrs of incubation.

***Rearing of Lavae***
During the first 3 days, the healthy larvae were nourished by the yolk deposit and subsequently fed with artemia from the 4th day for 2 weeks at 3% body weight.

***Rearing of Fingerlings***
A total of seventy five (75) fingerlings each from cold shocked and control groups were stocked in the culture receptacle (plastic tanks 1m x $1m^3$) and fed artificially with extruded feed for a rearing period of 14 weeks at 3% body weight. Weight and length measurement was done on a weekly basis. The remnant feed in the culture receptacle was siphoned out to prevent water fouling and mortality of fingerlings.

***Growth Indices***

$$\text{Mean weight gain (\%)} = \frac{\text{Final mean weight} - \text{Initial mean weight}}{\text{Initial mean weight}} \times \frac{100}{1}$$

$$\text{Mean length gain (\%)} = \frac{\text{Final mean length} - \text{Initial mean length}}{\text{Initial mean length}} \times \frac{100}{1}$$

$$\text{Specific Growth Rate} = (\text{SGR}) (\%\text{day}^{-1}) = \frac{100(\log \text{Final weight} - \log \text{Initial weight})}{\text{Time (t) in days}}$$

$$\text{Survival rate (SR) (\%)} = \frac{\text{Total No of Fish harvested}}{\text{Total No of Fish stocked}} \times \frac{100}{1}$$

$$\text{Hatchability rate (HR) (\%)} = \frac{\text{Total No of eggs fertilized}}{\text{Total No of eggs hatched}} \times \frac{100}{1}$$

The data on red blood cells analysis and erythrocyte measurement were analysed using duncam multiple wide range test.

**Results**
Erythrocyte measurement of (2ml of blood were extracted from the fish) and (two) drop of the blood was smeared on a pre-cleaned micro slide. The blood smear was allowed to air dry for 10-15 minutes and was stained with 15% Giemsa for 45 minutes. Blood smear was washed with distilled water and allowed to air dry for 10-15 minutes. It was again washed with Xylem for 10minutes. Cover slide was used to cover the smear after (added with drops of 2-3 DPX (Dibutyl Phthalate Xylem). A binocular microscope using eyepiece micrometer was used to view the blood smear preparation at (1000X objective which was magnified to x1000) using the photomicrogram. Sizes of the cells and their nuclei were computed using the following formulae given by Felip *et al*. (2001a). the total volume of the red blood cell (RBC) is the major axis of the erythrocyte while the entire size of the nucleus is the minor axis.

$V_{erythrocyte} = 4/3 \times (A/2) \times (B/2)^2$

$V_{nucleus} = 4/3 \times (a/2) \times (b/2)^2$

Where:
A = major axis of erythrocyte
B = minor axis of erythrocyte
a = major axis of nucleus
b = minor axis of nucleus

**Discussion**
The red blood cells of tetraploid is bigger in sizes than triploid and diploid in the current study an indication that the chromosomes had really reflected blood crops of the cells.According to Felip, Piferrer, Zanu, and Carillo, (2001a), triploid and tetraploid fish exhibit 50% or more DNA per cell and total cell volume in triploids and tetraploid increase significantly in comparison to that of diploids to accommodate the extra amount of chromosomes. According to Ihssen *et al.* (1990) and Benfey (1999), in polyploid specimens, the size of the entire cell increases with increasing nucleus, which is associated with cells in the particular tissue. In particular, erythrocyte cellular and nuclear measurements

are proportional to ploidy in *H.longifilis and H.bidorsalis in this study*. On this basis, erythrocytes in fish show an ellipsoidal shape, erythrocyte cellular and nuclei size have been used as the criterion to identify polyploid fish by several researchers such as (Wolters, Libey, and Chrisman, 1982; Benfey, M. Sutterlin. 1984; Felip, Piferrer, Zanu, and Carrillo 2001a and Cal, Vidal, Camacho, Piferrer, and Guitian, 2005). Venkatachalam *et al.,* (2012) reported that the extent of triploidy was measured through Erythrocytes Nucleus Volume (ENV). In diploid fish, C. batrachus the ENV was reported to be 11.7±1.7μm$^3$ where as in triploid fish it was 17.2±3.2 μm$^3$. In association with increased Erytrocyte and *Hetrobranchus* with increased ploidy level of (*C. auratus)*. According to Graham et al. (1985) in *Salmo salar* the size content in the blood of triploids gained more than those in diploids.

## CONCLUSION

This study shows that triploidy and tetraploidy could be induced in *H.longifilis and H.bidorsalis* by cold shock treatment, 3 minutes after fertilization at 5$^0$C for 20 minutes while tetraploidy could be induced by administering cold shock treatment, 30 minutes after fertilization at 5$^0$C for 10 minutes. This study has also shown that erythrocyte measurement can be used to ascertain ploidy level in *heterobranchus longifilis* and *heterobranbranchus bidorsalis*.It was observed from the erythrocyte measurement that the red blood cells (RBC) in tetraploid fish were larger than red blood cell of both triploid and diploid fish.

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