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Agbebi O. T.

Department of Aquaculture and
Fisheries Management Federal
University of Agriculture,
Abeokuta, PMB 2240 Abeokuta,
Ogun State, Nigeria.

Chromosome set manipulation in *Heterobranchus longifilis* for growth performance

Agbebi O. T.

Abstract

Triploidy was induced in *Heterobranchus longifilis* by injecting the brooders with ovaprim for 15 hours latency period. The fertilized eggs were subjected to chromosome studies, after giving cold shock treatment at 5 °C for 40 minutes. Diploid and triploid juveniles raised with mean weight of 32.55 g and 42.40 g respectively were reared in 2 x 2 x 1.2 m³ outdoor concrete tanks. The treatments were in triplicates and were fed with diets containing 45% crude protein. At juvenile stage to nine months of age, triploid *H. longifilis* growth was significantly different ($p < 0.05$) having (mean weight 980 g), significantly heavier than the diploid counterparts (mean weight 788 g). Histological examination revealed that both diploid and triploid fish had well-developed genitalia, but no other sexual characteristics could be seen in the triploids. The comparative hematological parameters showed that diploid would respond to stress and injuries more than triploid. Dietary requirements showed that the triploids converted feed efficiently (FCR=1.05) than the diploid (FCR=1.95). Result of weight gain in triploid gave clear evidence that triploids performed better than diploids and this confirmed that triploids are larger than their diploid strains and may provide greater profits in commercial catfish culture than the diploids.

Keywords: Growth, Survival, Triploid, diploid, Aquaculture potentials

1. Introduction

Genetic biotechnologies in aquaculture focus primarily on increase growth rate, but it could also address increased disease resistance and increased environmental tolerance of simple techniques, such as hybridization to transfer specific genes between species. The improvement in knowledge of breeding requirements and the ability to artificially induce breeding through the administration of natural or synthetic hormones and/or environmental manipulations had been key factors that had facilitated the application of more advanced biotechnologies [5]. Selective breeding, and the maintenance of other stocks genetically improved by chromosome manipulation, line crossing, or sex reversal depend on controlled breeding of the farmed species [8].

Chromosome number in a species normally remains constant through successive generations and these results into constancy of characters [6]. The possible cause of variation in chromosome number is the failure of normal mitotic division [2]. Ploidy individuals have three or more complete sets of chromosomes instead of the usual two. This can be achieved through chromosome manipulation. The general characteristics of this phenomenon are that nucleus size and cell size increases due to the extra chromosomes (Tave 1991). The motives behind triploidy induction in fishes is an attempt at improved qualities. Triploids are created for growth and sterility. Gametes that are produced are usually aneuploid (Tave 1991; Wang *et al.*, 1998) [14].

Animal haploid chromosome number is a fundamental property of the organism's genetic make-up. It represents the minimum number of blocks of genes that remain linked together and segregate together at meiosis (Olorode *et al.* 1982; Griffiths *et al.* 2004) [6]. They emphasized that a particular taxonomic group of animals may have quite a wide range of chromosome numbers from one species to another.

The genus *Heterobranchus* is similar in many respects to *Clarias* but can readily be differentiated from the latter by the rayed dorsal fin followed by an adipose fin. Like *Clarias*, they have four pairs of barbels on their flattened, strongly depressed head (Yisa and Olufeagba, 2005) [16]. They live in the swamps and rivers, but prefer the former habitat.

Correspondence

Agbebi O. T.

Department of Aquaculture and
Fisheries Management Federal
University of Agriculture,
Abeokuta, PMB 2240 Abeokuta,
Ogun State, Nigeria.

Heterobranchus are caught with nets, traps, fish fences and on longlines, chiefly on the receding floods. They are very common and form a significant part of the commercial catch, particularly in the areas where there are many swamps. The flesh is less oily than the *Clarias gariepinus*. They are highly prized and they fetch higher prizes. *Heterobranchus* are noted to have well-developed gonads between June and September (Reed, *et. al*, 1982; Shinkafi and Ipinjolu, 2012) [12].

2. Materials and Methods

Pure breed of *Heterobranchus longifilllis* parental were subjected to artificial propagation with the latency period of 15 hr. The diploids were transferred into four aerated hatching tanks for incubation. The timing of both meiosis and mitosis cleavage (when the zygote divides to become a 2-celled embryo) was adequately observed before the triploid production were subjected to chromosome set manipulation. This was carried out by cold shocking the fertilized eggs in a cold regulated chamber at 5 °C for 40 mins prior the transferred into the well aerated hatching troughs on kakabans serving as egg collector in the incubator. The two strains were fed with zooplankton for 10 days, after the absorption of their yoke. Thereafter they were reared with 45% crude protein formulated diet twice daily. Mean weight and mean length of

diploid and triploid at hatching were taken. The survival rates of hatchlings were expressed as a percentage of the hatching rate.

At nine months, the adult triploid and diploid fish (*H. longifilllis*) were subjected to histological study. Diploids and triploids fish used in this study were full sibling. Hematology study was accessed to check the health status of the strains.

Ten diploids and triploids were sacrificed, and the ovaries and testes from each group were removed and fixed in Bouin's fluid for histology study [9]. Tissue samples were dehydrated in an alcohol series and mounted in paraffin; sectioned to a thickness of 6µm and stained with hematoxylin and eosin. Slides were observed through a Zeiss photomicroscope and photographed on Panatomic X film developed in Microdol. Physico-chemical parameters of the water during the experimental period were monitored. Growth data obtained were subjected to one-way analysis of variance (ANOVA) test ($P < 0.05$) and a multiple-range test was applied to characterize the differences between the treatments.

The study therefore, is to assess the aquaculture potentials of *H. longifilllis* species through the comparative growth analyses of diploid and triploid progenies.

3. Results

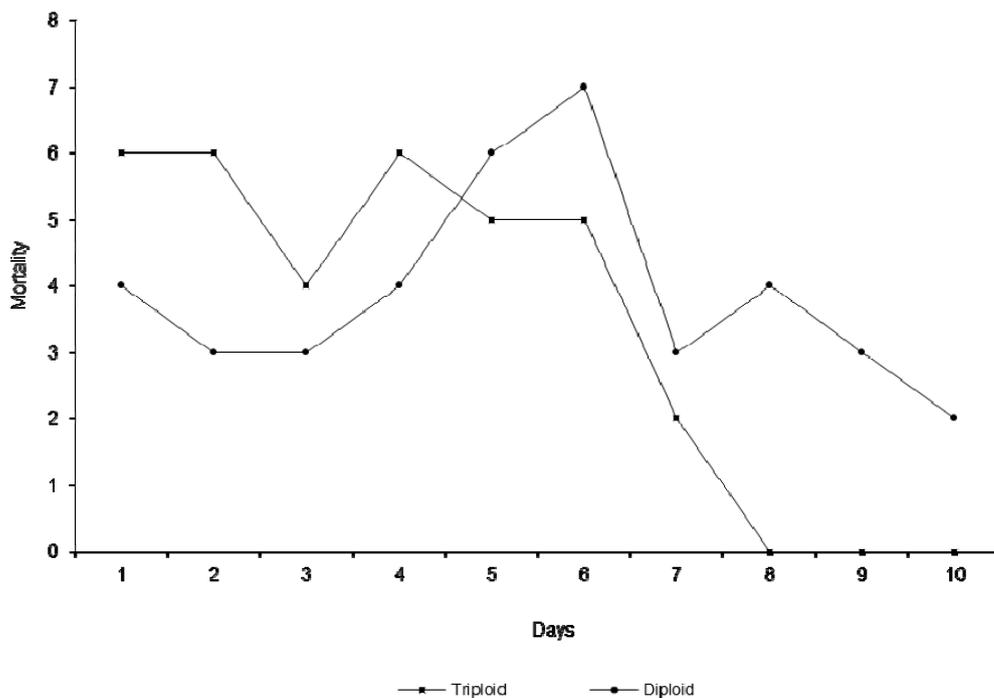


Fig 1: Daily Mortality of rate of Triploid and Diploid *Heterobranchus bidorsalis* fry raised in glass aquaria.

Table 1: Growth performance of triploid and diploid *Heterobranchus longifilllis* reared indoor tanks

Parameters	Diploid	Triploid	Significance
Initial Mean Weight (g)	42.64 ± 39.42	57.46 ± 64.72	0.001*
Final body weight (g)	75.56 ± 59.18	94.78 ± 87.66	0.001*
Relative weight gained (%)	43.57 ± 21.62	39.38 ± 29.02	0.001*
Daily weight gained (g)	0.27 ± 01.14	0.31 ± 03.07	0.669 ^{NS}
Specific growth ratio (%/day)	0.48 ± 0.81	0.42 ± 0.79	0.624 ^{NS}
Feed conversion ratio (FCR)	1.05 ± 0.71	1.95 ± 0.66	0.001*
Feed intake	10.30 ± 11.84	15.45 ± 17.53	0.001*
% Survival	92	100	0.001*

* Significant at 1% level; (NS) Not Significant

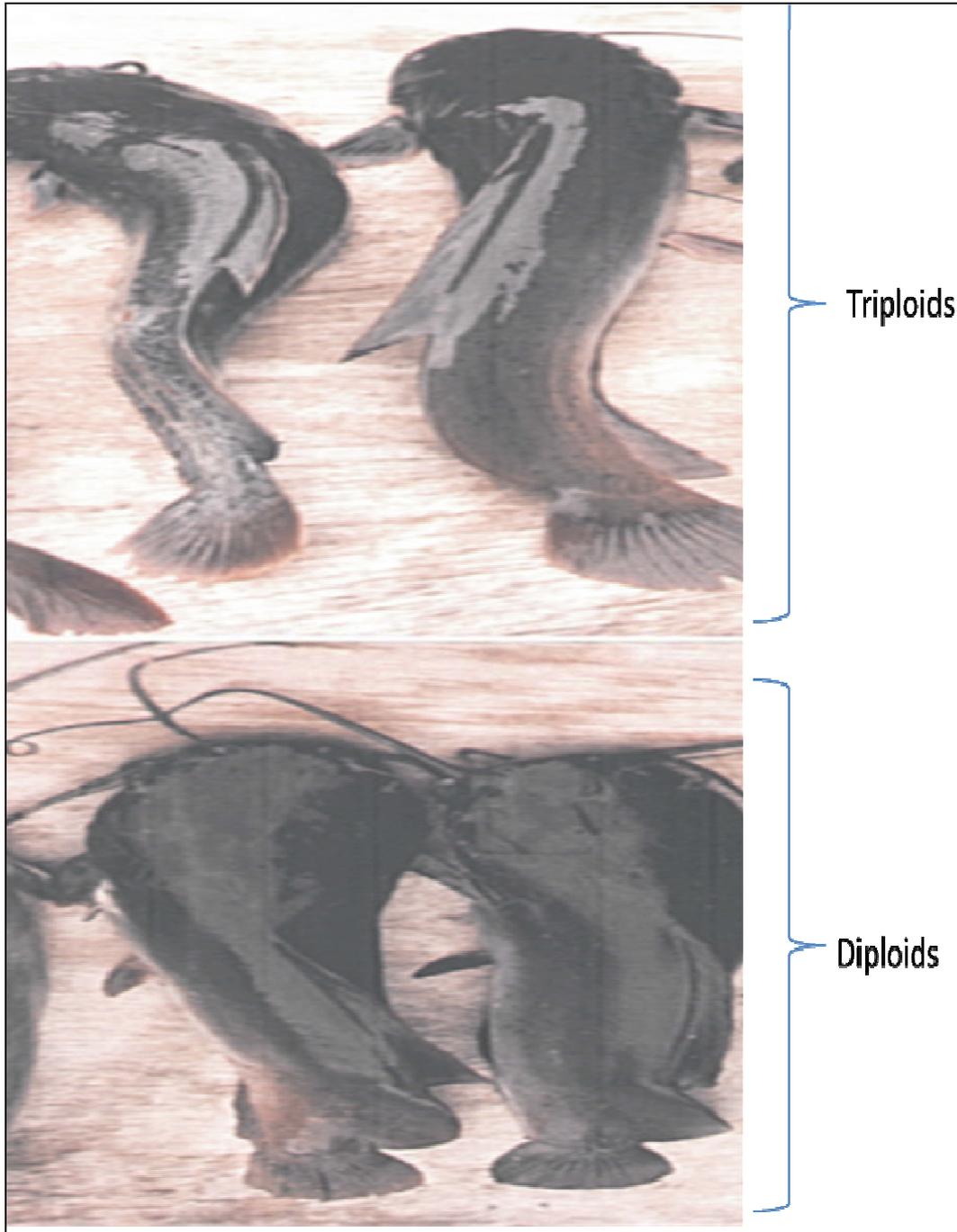


Plate 1: Diploids and Triploids at nine months.

Table 2: Haematological parameters of diploid and triploid *Heterobranchus longifilis*.

Parameters	Diploid	Triploid
PCV(%)	26.50 ± 2.45	33.44±7.40
Hb (mg/dl)	8.97 ± 0.68	10.62 ± 2.25
RBC (x10 ³ /μl)	2.49 ± 0.58	3.21 ± 0.78
WBC (n/μl)	19069 ± 665.56	18092 ± 520.90
Platelet	1000044± 1629.36	94622 ± 13272.90
MCV (Fl)	109.25 ± 18.44	106.59 ± 8.27
MCHC	35.50 ± 0.84	32.10 ± 0.49
Lymphocytes (n/μl)	73.33 ± 2.70	67.64 ± 3.98

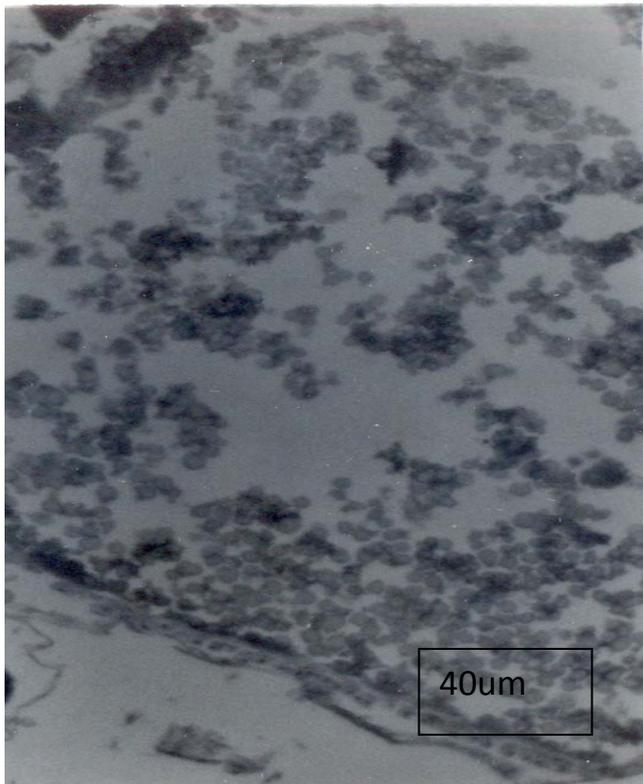


Plate 2A: Testis of triploid *Heterobranchus longifilis* (absolutely empty seminiferous tubules, only connective tissues frame work evident)



Plate 2B: Ovary of triploid *Heterobranchus longifilis* (watery ova)

Plate 2: Comparisons of histological sections of testis and ovary of triploid *Heterobranchus longifilis*

Table 3: Summary of water quality parameters during the experiment

Parameters	Range	Mean
Temperature (°C)	26.00- 28.85	27.32 ± 0.90
Dissolved Oxygen (mg/l)	6.60 – 8.00	7.90 ± 0.64
Conductivity (µs/cm)	76.10 – 83.10	79.60 ± 3.21
pH	6.22 – 6.86	7.22 ± 0.57
Ammonia (mg/l)	0.05 – 0.09	0.07 ± 0.02

4. Discussion

The triploids experienced low hatchability and survival at the incubation period due to cold shocking effect on their eggs. After the initial low survival rate of triploid 50% as against 74% for diploid, survival rate was improved and stabilized on the eighth day in the incubator (Fig 1).

The strains were restocked for an indoor rearing and triploid *H. longifilis* hatchlings remained stable under intensive management because only very good and viable eggs could survive the temperature shock. The growth rate from hatching to the 6th week of rearing was 0.044±0.02 to 4.5±0.666 in triploid and 0.040±0.12 to 3.6±0.549 in diploids. The difference in the means was 4.46 g and 2.56 g in triploid and diploid respectively within the growing period. Percentage survival of both diploid and triploid were equal during the growing stage in the aquaria tanks (Table 1). The reason for better survival could be attributed to the initial hardness of shocking effect triploid has undergone. Once certain triploid population survived the shock and the stress, they could therefore, withstand other environmental and health hazard [1]. The two strains were reared to nine months in outdoor experimental concrete tanks. However, triploids maintained 100% survival throughout the experimental period, and

diploid, 92% survival was recorded. Generally Diploid males are darker in colouration than female diploid. Triploid fish are brighter in body colouration than diploid fish. Triploids are longer in length while diploids are study (plate 1)

This result corroborated with the findings of (Wolters *et al.* 1982b) [15], Channel catfish triploids become larger than diploids at about nine months of age when grown in tanks. In tank experiments, the triploids converted feed more efficiently than diploids. This is in support of Wolters *et al.* 1982a, Chrisman *et al.* 1983 and Dunham *et al.*, 2000 [3, 4, 15] triploids had six percent greater carcass yield at three years of age and were darker than diploids. The only explanation for this growth difference of triploid over diploid can be attributed to increase in cell volume. Triploid cell contained three sets of chromosomes instead of the normal two sets [6]. Tave (1992) [13] stated that fish eggs do not extrude the second polar body until they are fertilized. When a newly fertilized egg is subjected to temperature shock, the shock prevents the second polar body from leaving the egg. Consequently, the fertilized egg will contain three haploid nuclei.

The histology examination observed in the triploid females showed undeveloped ovaries, threadlike, with immature eggs; while the diploids had well developed ovaries at the phase of gonad development (Plate 1 & 2). This suggested high fertility in the diploids while the triploids tended to be sterile. The histology examination observed diploid *H. longifilis* showed evidence of gonad development at nine months of age. The lack of gonad development in triploids provides an explanation for the better feed conversion, success in chromosomal manipulations, significantly larger weight and sterility. This study is agreement with Benfey 1999 who observed severe gametogenetic impairment in Salmon triploid fish individuals.

Variations in the haematological values of the diploids and triploid were very minimal. There were positive correlations among PCV, HB, RBC and WBC at 5% level of significance. The above observation reveals that the blood components of diploid and triploid *H. longifilis* were apparently healthy. Olufeagba (1999) corroborated these findings in *H. longifilis*. The survival rate obtained in this result implies that the ranges of water quality parameters were within the tolerable level for the successful culture of the strains

5. Conclusion and Recommendation

Maturity was attained at nine months of intensive culture in concrete tanks which shows that these species could attain sexual maturity three months earlier compared to thirteen months of sexual maturity reported earlier for the same species by Otema *et al.* (1996), (Gupta and Acosta, 2004) ^[7, 11]. The early maturity of triploid strain could be due to its ability to convert feed more efficiently than diploid strain.

The result of this study revealed that triploids survived better than their diploid counterparts and no particular difficulty was experienced in rearing the triploid fishes up to maturity. Chromosome set manipulation should be encouraged in hatcheries as helping tools for fish farmers for multiple harvest periods, weighty fish stocks and maximize profit.

The triploid production in this study was cold shock method. It is suggested that further research be carried out on the warm shock technique as a combined or substitute for other chromosomal manipulation.

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