

Growth Performance and Gonad Development in Diploid and Triploid *Clarias batrachus* (Linnaeus)

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ABSTRAK

Sibling penuh ikan keli kayu, *Clarias batrachus*, diploid dan triploid dipelihara dalam tangki gentian kaca segi empat mulai umur tiga minggu. Prestasi pertumbuhan ikan diploid dan triploid dibandingkan dengan diberi makanan yang mengandungi 30% protein. Pada akhir tempoh ujikaji ini kadar pertumbuhan di antara diploid dan triploid didapati tiada perbezaan bererti ($P>0.05$). Walau bagaimanapun gonad ikan triploid tidak begitu berkembang. Ovari ikan triploid mengandungi bilangan oosit yang kurang dan beberapa oosit matang yang abnormal. Testis ikan triploid mengandungi bilangan tubul seminiferus yang kurang dan jumlah tisu perantara yang bertambah.

ABSTRACT

Full siblings of diploid and triploid walking catfish, *Clarias batrachus*, were reared in rectangular fibreglass tanks starting at the age of three weeks. Growth performances of diploids and triploids were compared by feeding with a 30% protein diet. At the end of the study period growth rate was found to be insignificantly different ($P>0.05$) between the diploid and the triploid. However, triploid fish had poorly developed gonads. Triploid ovaries contained fewer primary oocytes with some abnormal maturing oocytes. Triploid testes contained fewer seminiferous tubules and a larger amount of connective tissue.

Keywords: diploid, triploid, growth, gonad development

INTRODUCTION

The catfish, *Clarias batrachus* and *C. macrocephalus*, are very popular and constitute some of the most important freshwater fish in Malaysia. They are utilised not only for fish farming but also for inland fisheries. They attain a size of above 200 g after four to six months of culture in earthen ponds. In Malaysia spawning induction in walking catfish has been described by Thalathiah (1986) and Cheah *et al.* (1990) but there is no information on chromosome manipulation work on this fish. Triploidy has been successfully achieved in *C. macrocephalus* and *C. gariepinus* (Richter *et al.* 1987; Vejaratpimol and Pewnin 1990). Recently, triploid *C. batrachus* were produced by cold-shocked treatments of the inseminated eggs (Manickam 1991; Siraj *et al.* 1992). The interest is especially focused on triploidy, as triploid fish have been assumed to be sterile and potentially can avoid the growth depression, poorer feed conversion and survival, losses which are associated with sexual maturation

in normal fish (Purdom 1976; Thorgaard and Gall 1979; Gervai *et al.* 1980).

Gonad development and fertility in induced triploidy and higher degrees of ploidy are known for a number of amphibian species (Frankhauser and Humphrey 1950; Bungenberg De Jong 1957), but comparatively few studies have been made on fish, probably because of the difficulties of inducing and rearing polyploid fry. Experimentally-induced triploids have been reared in a variety of fish (Swarup 1959a,b; Purdom 1972; Valenti 1975; Wolters *et al.* 1982; Chourrout *et al.* 1986). In some of these species polyploidy appeared to be associated with a marked disruption of gonad development; either lacking or undeveloped, although some fish within the normal diploid range for nuclear cell were also sterile. Thus, triploids might be more valuable and profitable to raise than diploids.

This study was conducted to examine the effects of triploidy on the growth performance and gonad development of *C. batrachus*.

MATERIALS AND METHODS

Three-week-old full siblings of triploids (obtained from cold shock treatment inseminated eggs immersed in water at 5°C and 10°C, for a duration of 3, 5 and 10 min and assessed by the size of erythrocytes major axis (Siraj *et al.* 1992)) and normal diploids were reared in 500 l rectangular fibreglass tanks containing 200 l of water, equipped with biological filters and aeration. Fifty fish were stocked in each tank with three replicates per treatment (both diploid and triploid). Fish were fed with a 30% protein diet two times a day and uneaten feed was siphoned out. Water quality parameters, such as temperature, pH, dissolved oxygen, carbon dioxide and nitrite were monitored during the experimental period. The rearing experiment was terminated at the end of five months since more than 50% of the fish died. All fish were weighed to the nearest 0.1 g. Feed conversion was calculated from the weight of feed consumed divided by the weight of fish produced (wet weight). Growth performance and feed conversion rate were analysed using statistical programme.

At the end of ten months ovaries and testes from five females and five males from each group were removed and immediately fixed overnight in Bouin's solution. The gonads were dehydrated in a series of alcohol and mounted in paraffin wax and stained following Drury and Wallington (1980). Transverse sections from both ends of each gonad were cut at 5-6 µm in thickness and stained with hematoxylin and

counterstained with eosin. Slides of both ovary and testes from diploid and triploid *C. batrachus* were examined for their cell structure and stages, and photographed.

RESULTS

Table 1 gives mean total length and weight, and feed efficiency of diploid and triploid *C. batrachus* from three weeks to five months of age. At 5 months old the values were 148.09±2.05 mm, 26.40±1.24 g and 1.47 for diploid fish and 147.60±2.25 mm, 25.91±1.25 g and 1.44 for triploid fish, respectively. There was no significant difference ($P>0.05$) between diploid and triploid *C. batrachus* in weight, total length and feed conversion rate up to five months of growth. Water quality parameters such as temperature, pH, dissolved oxygen, carbon dioxide and nitrite were within the physiological conditions.

The bilobed testes of diploid walking catfish at an age of ten months were elongated and the posterior end of each lobe was fused to form a single *ductus efferens*. The anterior region of the testes contained a series of seminiferous tubules where spermatogenesis takes place. Reduced size of the posterior end of the testes suggests that fewer seminiferous tubules develop in this region. General structure of the triploid walking catfish testes (Plate 1B) was almost similar to that of diploid (Plate 1A) except that the size was smaller, and coiled seminiferous tubules at the anterior region were fewer and irregular in shape.

TABLE 1.
Mean (\pm SD) weight, total length and feed conversion rate for diploid and triploid walking catfish *Clarias batrachus* at 5 months.

Variable	Diploid		Triploid	
	Mean weight (g) \pm SD	Mean total length (mm) \pm SD	Mean weight (g) \pm SD	Mean total length (mm) \pm SD
3 weeks	2.10 \pm 0.90	60.98 \pm 1.01	1.65 \pm 0.06	57.07 \pm 0.71
5 months	26.40 \pm 1.24	148.09 \pm 2.05	25.91 \pm 1.25	147.60 \pm 2.25
Feed conversion ratio (at 5 months)		1.47		1.44

A histological section of testes from diploid catfish revealed the seminiferous tubules were encircled by spermatogenesis epithelium of the same thickness. The lumen of tubules were filled with many spermatozoa (Plate 2A). In the triploid testes there was irregular thickness of the spermatogonia epithelium, and an increased amount of connective tissue (Plate 2B). This suggests that a great proportion of the spermatocytes and

spermatids degenerate. Similar observations were reported by Lincoln (1981) and Wolters *et al.* (1982) in plaice and channel catfish respectively.

The size of triploid ovaries was about one-fourth of the diploid ovaries (Plate 3). Various stages of developing oocytes were observed in the histological section of the diploid ovaries (Plate 4A). Strong basophilic cytoplasm and light stained nuclei were found in immature and

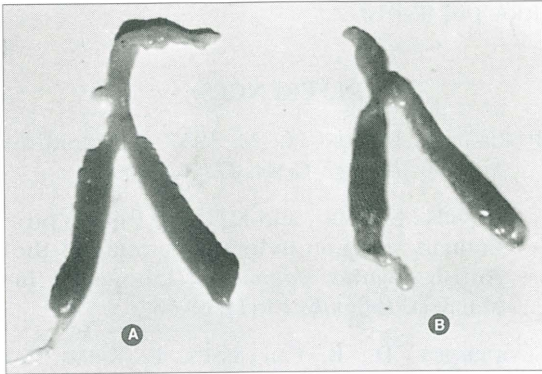


Plate 1 Diploid (A) and triploid (B) testes of walking catfish, *Clarias batrachus* (2x) at 10 months.

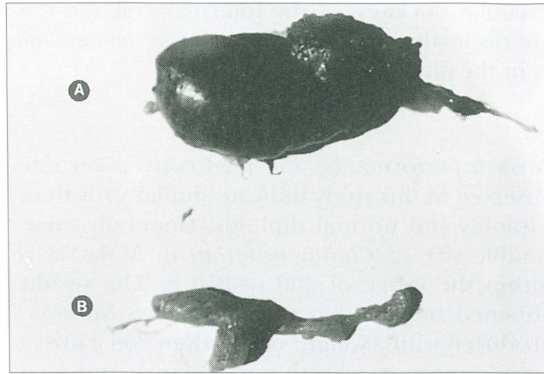


Plate 3 Ovaries of diploid (A) and triploid (B) of walking catfish *Claris batrachus* (2x) at 10 months.

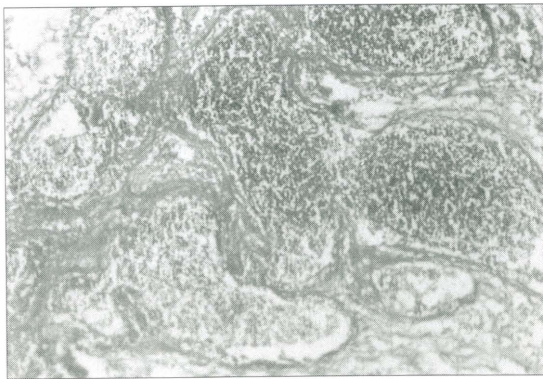


Plate 2A Histological section of testes of diploid walking catfish *Clarias batrachus* (200x)

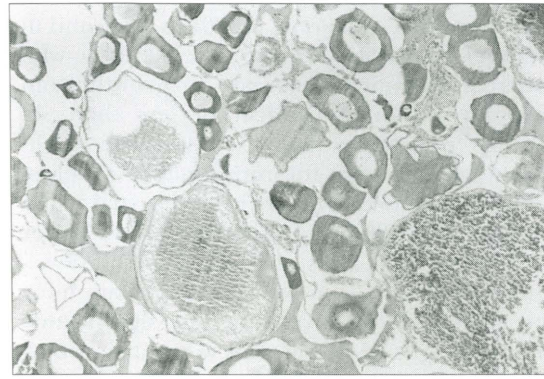


Plate 4A Histological section of ovary of walking catfish *Clarias batrachus* (400x)

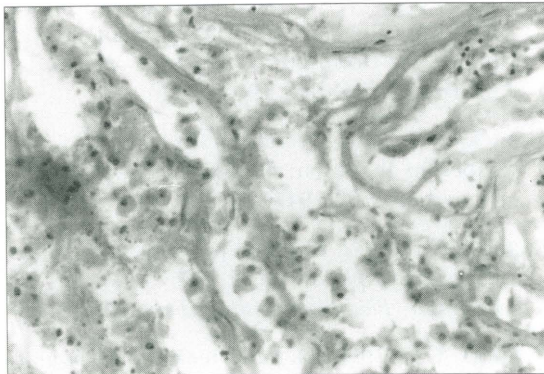


Plate 2B Histological section of testes of diploid walking catfish *Clarias batrachus* (400x)

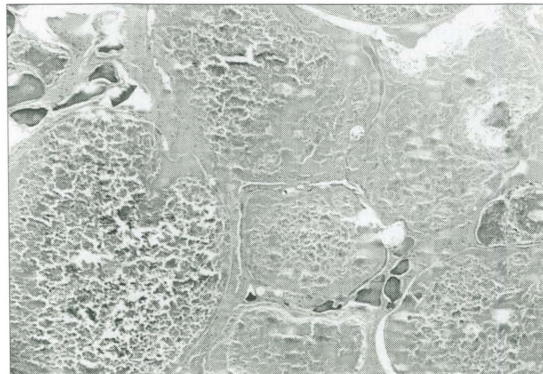


Plate 4B Histological section of ovary of triploid walking catfish *Clarias batrachus* (400x)

maturing oocytes of the diploid catfish. In contrast the triploid ovaries had fewer oocytes in different developmental stages. Many of the maturing oocytes had poorly defined nuclear membranes and highly granular cytoplasm which was different from the normal stage VI oocyte in the diploid ovaries. Few primary oocytes were also present in the ovary of the triploid catfish (Plate 4B). The triploid oocytes showed an irregular thickness of the follicular cell and was not distinctly differentiated from the *zona radiata* as in the diploid catfish.

DISCUSSION

Growth performance and feed conversion rate observed in this study indicate similar growth of triploids and normal diploids. Generally, marketable size of *Clarias batrachus* in Malaysia is within the range of 200 to 250 g. The results obtained suggest that triploid *Clarias batrachus* produced with a weight of less than 200 g are at no advantage in growth performance and feed conversion rate. The advantage could be felt if the marketable weight is more than 500 g at ten months. These results were in agreement with work done by Gervai *et al.* (1980) in carp, and in channel catfish by Wolters *et al.* (1982) who found no significant increase in growth from triploidy in immature fish of less than six months' growth. In theory, triploid fish would expend less energy for reproductive development during the normal period of sexual maturation than diploids and therefore show better feed conversion. Thus, the lack of gonad development in triploids provides an explanation for the better feed conversion and significantly greater weight. However, this was not shown in the present study, probably due to the shorter period of rearing and the marketable size of the fish which is less than 200 g. The triploid males which produced gonads of about the same size as in diploids give no advantage in term of growth performance and feed conversion rate. Further repeated experiments are required to verify the theory as proved by studies on plaice-flounder hybrids, *Pleuronectes platessa* X *Platichthys flesus*, in rainbow trout, *Salmo gairdneri*, and in channel catfish, *Ictalurus punctatus* (Purdom 1976; Thorgaard and Gall 1979; Wolters *et al.* 1982). Further research is also needed to look into the economics of producing triploid and other chromosomal manipulated walking catfish which could be of greater value to the fast-developing fisheries industry in the country.

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REFERENCES

- BUNGENBERG DE JONG, C. M. 1957. Polyploid in animals. *Bibliogr. Genet.* 27.
- CHEAH, S.H., S.S. SIRAJ and K.J. ANG. 1990. A preliminary study on induced spawning of the catfish *Clarias batrachus* (Linnaeus) in Malaysia. *Pertanika* 13 (1): 63-66.
- CHOURROUT, D., B. CHEVASSUS, F. KRIEG, A. HAPPE, G. BURGER and P. RENARD. 1986. Production of second generation triploid and tetraploid rainbow trout by mating tetraploid males and diploid females - Potential of tetraploid fish. *Theor. Appl. Genet.* 72: 193-206.
- DRURY, R.A.B. and E.A. WALLINGTON. 1980. *Carleton's Histological Technique*. 5th. ed. Oxford: Oxford University Press.
- FRANKHAUSER, G. and R.R. HUMPHREY. 1950. Chromosome number and development of progeny of triploid axolotl females mated with diploid males. *J. Exp. Zool.* 115: 207-249.
- GERVAI, J., S. PETER, A. NAGY, L. HORVATH and V. CSANYI. 1980. Induced triploidy in carp, *Cyprinus carpio* L. *J. Fish Biol.* 17: 667-671.
- LINCOLN, R.F. 1981. Sexual maturation in triploid male plaice (*Pleuronectes platessa*) and plaice X flounder (*Platichthys flesus*) by hybrids. *J. Fish. Biol.* 19: 415-426.
- MANICKAM, P. 1991. Triploidy induced by cold shock in the Asian catfish, *Clarias batrachus* (L.). *Aquaculture* 94: 377-379.
- PURDOM, C.E. 1972. Induced polyploidy in plaice (*Pleuronectes platessa*) and its hybrid with the flounder (*Platichthys flesus*). *Heredity* 29: 11-23.
- PURDOM, C.E. 1976. Genetic techniques in flatfish culture. *J. Fish. Res. Board Canada* 33: 1088-1093.

- RICHTER, C.J.J., A.M. HENKEN, E.H. EDING, J.H. VAN DOESUM and P. DE BOER. 1987. Induction of triploidy by cold-shocking eggs and performance of triploids of the African catfish, *Clarias gariepinus* (Burchell, 1822). In *Proc. World. Symp. On Selection, Hybridization, and Genetic Engineering in Aquaculture*, Bordeaux 27-30 May, 1986. Vol. II. Berlin.
- SIRAJ, S.S., Y.K. LAW, S. H. CHEAH and K. J. ANG. 1992. Induced triploidy in walking catfish, *Clarias batrachus* (Linnaeus) by water temperature shock. *Malays. Appl. Biol.* **21**(1): 1-5.
- SWARUP, H. 1959a. Production of triploidy in *Gasterosteus aculeatus* (L.). *J. Genet.* **56**: 129-142.
- SWARUP, H. 1959b. Effects of triploidy in the body size, general organization and cellular structure in *Gasterosteus aculeatus* (L.). *J. Genet.* **56**: 143-155.
- THALATHIAH, S. 1986. Induced spawning of *Clarias macrocephalus* (Gunther). In *The First Asian Fisheries Forum* ed. J.L. Maclean, L.B. Dizon and L.V. Hosillos, p. 683-686. Manila: Asian Fisheries Society.
- THORGAARD, G.H. and G.H. GALL. 1979. Adult triploids in a rainbow trout family. *Genetics* **93**: 961-973.
- VALENTI, R.J. 1975. Induced polyploidy in *Tilapia aurea* (Steindachner) by means of temperature shock treatment. *J. Fish. Biol.* **7**: 519-528.
- VEJARATPIMOL, R. and T. PEWNIN. 1990. Induction of triploidy in *Clarias macrocephalus* by cold shock. In *The Second Asian Fisheries Forum* ed. R. Hirano and I. Hanyu. p. 991. Manila: Asian Fisheries Society.
- WOLTERS, R.W., C.L. CHRISMAN and G. S. LIBERG. 1982. Effect of triploidy on growth and gonad development of channel catfish. *Trans. Am. Fish. Soc.* **111**: 102-105.

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