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(Accepted 29 December 1992)

Comparative Study on the Growth and Gonadal Development of Diploid and Triploid Tilapia, *Oreochromis aureus*

Abstract

Heat shock-induced triploid and diploid *Oreochromis aureus* were reared in hapas (2 × 1.2 × 0.9 m). Their growth rates were not significantly different (P>0.05) at 24 weeks old after hatching. The genital papillae of triploid fish did not show significant development in comparison with diploid fish. The gonadosomatic indices of female triploid fish were significantly smaller (P<0.01) than those of diploid fish. Non-uniform sizes of sperms were observed histologically in triploid fish. Well-accumulated yolk was pronounced in the oocytes of 24-week old diploid fish. On the other hand, most of the oocytes in triploid fish were oogonia.

Key words: *Oreochromis aureus*, Diploidy, Triploidy, Growth, Gonadal development

Triploidy often becomes functional sterility because the pairing of chromosomes during meiosis I is impeded by the presence of three homologous chromosomes resulting in the unequal or aborted separation of homologous chromosomes. Triploidy may enhance growth due to the sterility and altered cell physiology, especially after the age of maturity of the fish(1–4). Metabolic energy, normally utilized during gonadal development, may be available for the growth of triploid fish after maturity(5–6).

Tilapias are known for their precocious and strong reproductive ability before reaching market size. Thus the main goal of most research on tilapias is to produce all male or sterile fry. Several studies have already been done on the induction of triploidy in tilapias using cold and heat shock(7–11). Comparative studies on growth between diploid and triploid fish, however, are relatively few, with the results as inconsistent (Table 1). Valenti(7) reported that no significant differences in length or weight were found between 6-week old diploid and polyploid *O. aureus*. However, between 14-week old fish, all six polyploid fish examined were significantly larger than the diploid ones. Don and Avtalion(9) observed that there was no difference in both the morphology and growth rate between 6-month old diploid and triploid *O. aureus*. In the present study, the differences in morphology, growth, and gonadal development between diploidy and triploidy were studied.

Materials and Methods

The experiment was conducted at the Tungkang Marine Laboratory, Taiwan Fisheries Research Institute. A batch of 100% triploid *O. aureus* fry were induced at a heat shock treatment of 41°C(19). The fry were reared from feeding stage in the hapas (2 × 1.2 × 0.9 m) at a water depth of 60 cm in a wooden rectangle tank lined with plastic sheet. Triplicated hapas both in the diploid and triploid groups were held in the same wooden-plastic rectangle tank. Twenty fish were kept in each hapa.

The fry were fed twice a day with commercial eel feed in the first 12 weeks and commercial tilapia floating pellet feed thereafter for 12 weeks. The crude

Chang S. L., C. F. Chang and I C. Liao (1993) Comparative study on the growth and gonadal development of diploid and triploid tilapia, *Oreochromis aureus*. J. Taiwan Fish. Res., **1**(1): 43–49.

Table 1. Summary of growth and gonadal development studies between various diploid and triploid fishes.

Scientific Name	Growth Performance	Triploid Gonad Female	Triploid Gonad Male	References
Pleuronectes platessa X *Platichthys flesus*	2N=3N			Purdom (1972)[13]
Oreochromis aureus	3N>2N			Valenti (1975)[7]
Cyprinus carpio	2N=3N	retarded	retarded	Gervai et al. (1980)[14]
Ictalurus punctatus	3N>2N	retarded	retarded	Wolters et al. (1982)[1]
Oncorhynchus spp.	2N>3N			Utter et al. (1983)[15]
Salmo gairdneri	2N>3N	retarded	retarded	Solar et al. (1984)[16]
Crassostrea virginica	2N>3N[a] 3N>2N[b]			Stanley et al. (1984)[17]
Argopecten irradians	3N>2N	retarded	retarded	Tabarini (1984)[2]
Misgurnus anguillicaudatus	2N>3N(<1 yr) 3N>2N(>1 yr)	retarded	retarded	Suzuki et al. (1985)[3]
Oreochromis aureus	2N=3N	retarded	retarded	Don and Avtalion (1986)[9]
Silurus glanis	3N>2N	retarded	retarded	Krasznai and Marian (1986)[4]
Chlamys nobilis	2N>3N	retarded	retarded	Komaru and Wada (1989)[18]

[a] Triploidy induced from inhibiting meiosis II. [b] Triploidy induced from inhibiting meiosis I.
2N: Diploid 3N: Triploid

Table 2. Summary of growth in diploid and triploid *Oreochromis aureus* reared in hapas (2 × 1.2 × 0.9 m) for 24 weeks from the beginning of feeding stage.

Ploidy	No. of fish	Mean total length (cm) Initial	Mean total length (cm) Final	Mean body weight (g) Initial	Mean body weight (g) Final	Condition factor
Diploid	60	0.922±0.017	18.89±2.20	0.0086±0.0008	119.79±40.00	1.73
Triploid	60	0.934±0.034	18.41±0.91	0.0083±0.0008	109.50±21.70	1.73

protein content of the feeds were 44% and 23%, respectively. Water temperature was measured daily at 0830 and 1430. Underground water was used for the growth experiment. Water quality and algal density were kept to their optimum as possible. The fish were measured at 3-week intervals.

Twenty fish were randomly sampled and dissected from each treatment at the end of the experiment. After the body and gonadal weight were measured, histological studies were undertaken on the gonad. The dissected samples of gonad, which were immersed in 10% formalin for one or two days, were rinsed for 24 h under running water to remove the formalin. The samples were then processed sequentially in 70%, 80%, 90%, 95% (I), 95% (II), 100% (I), 100% (II) alcohol for 24 h, 2 h, 2 h, 1 h, 1 h, 8 h and 2 h, respectively.

To dehydrate and embed the samples, they were treated with xylene (I), xylene (II), xylene + paraffin and paraffin for 60 min, 45 min, 45 min, and 40 min, respectively . The samples were then sectioned into 5–8 μm by microton and stained with Mayer's hematoxylin and counterstained with eosin (HE)[20–21], and then, photographed. The gonadosomatic index (GSI) was calculated as:

GSI = (Gonad Weight / Body Weight) × 100

Results and Discussion

The mean water temperature was 26.6 ± 3.1°C during the experiment. There was no difference (P>0.05) between the body weight and total length of diploid and triploid fish before the initial feeding stage (Table 2). There was also no difference (P>0.05) between diploid and triploid fish at 24 weeks after hatching (Table 2 and Fig. 1). The variation in both the final total length and body weight was lower in triploid fish than in diploid fish. This indicates that the growth of triploid fish was more uniform than diploid fish. This finding is particularly advantageous in harvesting operations.

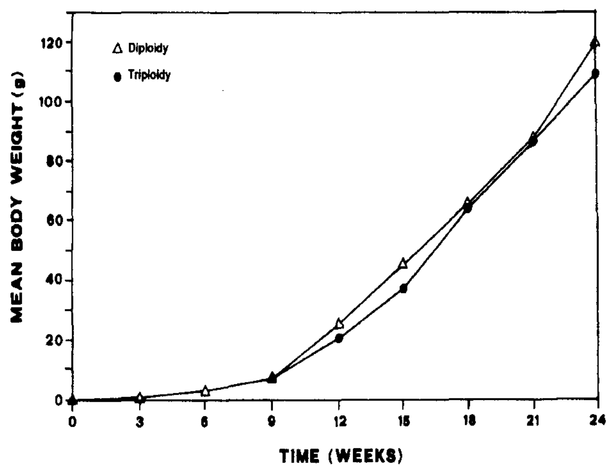

Fig. 1. Comparison of growth between diploid and triploid *Oreochromis aureus* reared in hapas (2 × 1.2 × 0.9 m) for 24 weeks.

No differences were also observed between the morphology of diploid and triploid fish within 9 weeks old. After 9 weeks, male diploid fish began to show their mating body coloration. The genital papillae of triploid fish showed no development in comparison with normal diploid fish from 18 weeks old. This result is consistent with the observation of Don and Avtalion[9]. Sexing diploid fish according to their genital papillae was easy, but difficult with triploid fish. In male triploid channel catfish, *Ictalurus punctatus,* no sexual characteristics were observed, thus it was difficult to sex female and male triploid fish externally[1]. Under histological section, the lumina of the tubules of triploid catfish were smaller and contained no spermatozoa at 6 months old.

Growth performance between diploid and triploid fish of various aquatic species was not consistent (Table 1). The different experimental methods to induce triploidy and the variable gene expressions could be important factors affecting growth. In this experiment, growth was not statistically significant between 6–month old triploid and diploid fish. But the growth rates of diploid tilapias should be impeded after the breeding. In loach, *Misgurnus anguillicaudatus,* mean body weights of triploid fish in the first year were lower in comparison with those of diploid fish. However, on the second year, weight of the triploid loach were greater than those in the control group of the diploid loach[3].

The mean body weight of female and male triploid fish were similar as opposed to diploid fish (Table 3). The fast growth of the diploid male compared to diploid female can be attributed to its voracity and aggressive behavior. The mean weight of the gonads of female and male diploid *O. aureus* was quite similar at the age of 24 weeks, but the gonad weight between female and male triploid fish were quite different (Table 3). Under histological study, well developed testes with numerous sperms were observed in 24–week old diploid *O. aureus* (Figs. 2–A, 2–B). Some test is of triploid fish also developed well with many non-uniform sperms that accumulated in the lobular lumen (Figs. 2–C, 2–D). Yolk accumulation was pronounced in the oocyte of the diploid fish (Fig. 2–E). On the other hand, most of the oocytes of the triploid fish were oogonia (Fig. 2–F) and only a few yolk–accumulated eggs were observed in the thread–like ovaries. In 6–month old triploid plaice, *Pleuronectes platessa,* few growing oocytes were also observed in the ovaries[13].

Homologous chromosome of male tripoid fish could be paired and separated successfully during meiosis as observed in the non–uniform sizes of the sperms. Some of the sperms provided possibly diploid and the other, haploid. Probability of success in the pairing and separation of homologous chromosome of female triploid fish is quite low in comparison with male triploid fish as observed in the extremely few yolk–accumulated eggs in some of the ovaries.

Table 3. Comparison of gonad weight and gonadosomatic indices (GSI) between 24–week old diploid and triploid *Oreochromis aureus.*

	Body weight (g)		*Gonad weight (g)*		*GSI*	
Ploidy	***Female***	***Male***	***Female***	***Male***	***Female***	***Male***
Diploidy	105.84±10.77	183.68±27.70	1.02±0.93	1.01±0.34	0.96	0.55
Triploidy	125.39±24.84	125.81±11.42	0.08±0.10	0.34±0.31	0.06	0.27
t–test			P<0.01	P<0.01		

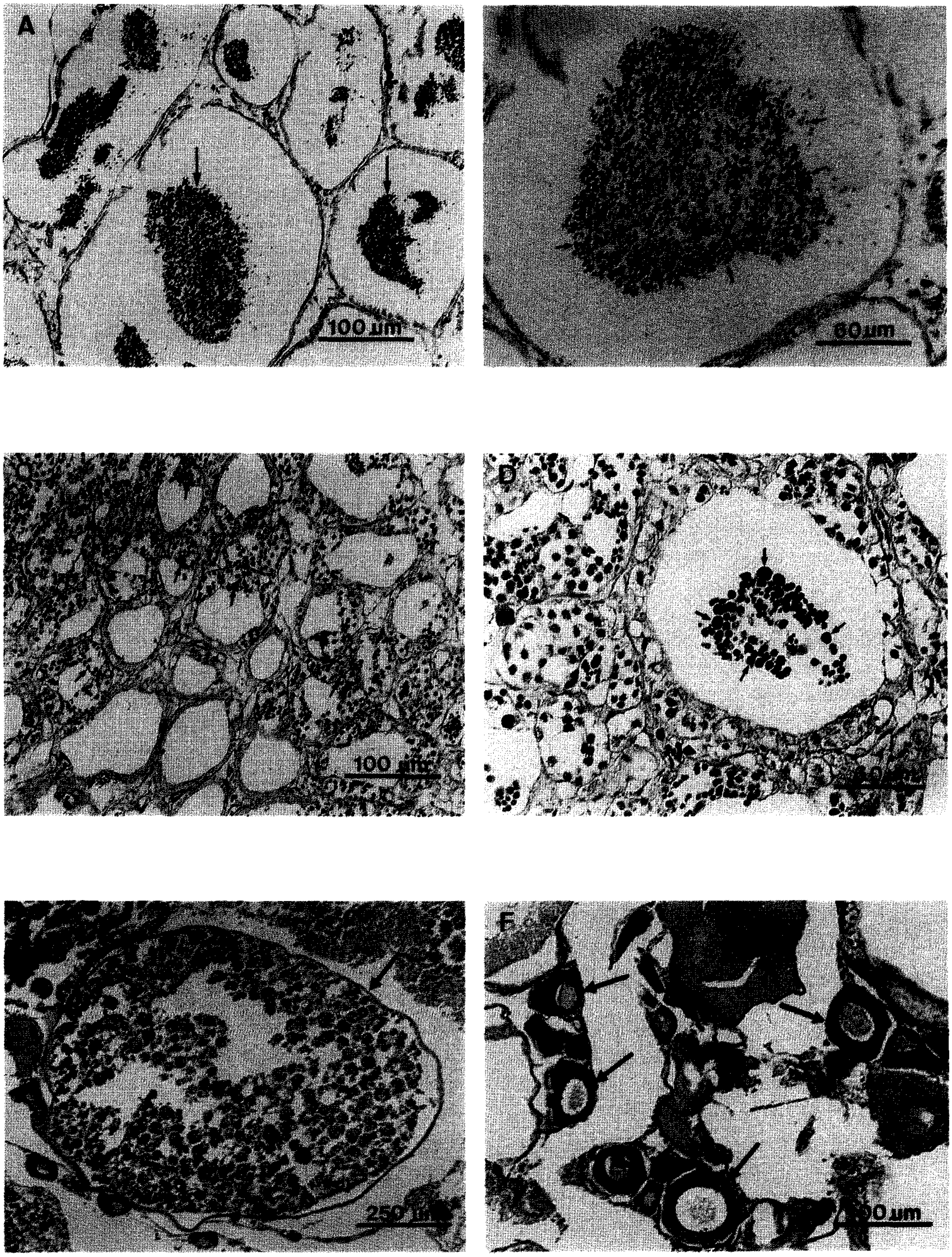

Fig. 2. Histological studies on the gonad of *Oreochromis aureus.* (A) and (B) male diploid gonad: numerous sperm in lobular lumen; (C) and (D) male triploid gonad: few sperm with non-uniform size in lobular lumen; (E) female diploid gonad: yolk well accumulated in oocyte; (F) female triploid gonad: oocytes are oogonia.

Hussain et al.[22] reported that triploid Nile tilapia, *Oreochromis niloticus,* sperms were mostly unable to fertilize normal eggs in 10 different crosses. Although only a few of the eggs were fertilized, the hatched larvae were found morphologically deformed and dead before yolk sac resorption. Through karyotypic analysis, the embryos from such crosses were all aneuploid .

Tilapia culture had its beginnings in Taiwan about 45 years ago when *O. mossambicus* was introduced as an exotic species in 1946. Soon it multiplied and eventually spread in almost all rivers, estuaries and Dapong bay, which is the only saline lagoon in Taiwan. Tilapias, because of their carnivorous nature, could be harmful to other freshwater and seawater fish fry. Some countries, notably in Nauru, are already experiencing widespread problems following the introduction of exotic tilapia species, such as a sharp decrease in pre–existing commercial native species populations. In the Buada Lagoon of Nauru, the reduced milkfish resources have been attributed to the widespread invasion of tilapia population in the lagoon. Introduction of tilapias should therefore be carefully evaluated. Otherwise, native species will be seriously impacted by the tilapias. Introduction of sterile fish for aquaculture and for controlling the aquatic weeds should be well controlled .

Because of their lower fecundity, triploid tilapia fry are difficult to produce commercially via retention of the second polar body from the fertilized eggs. To produce triploid tilapia fry by crossing a tetraploid with diploid fish seems a circumventing way. Chourrout et al.[23] found that triploid rainbow trout, *Salmo gairdneri,* fry produced by crossing tetraploid fish with diploid fish had a better growth rate than when produced via a retention of the second polar body from diploid fish. In tetraploid tilapias, however, the survival rate were quite low or not viable as in Don and Avtalion[10] and Myers[24]. More studies should be done along this line.

Acknowledgments

The first author would like to express his sincere gratitude to the National Science Council for financial support in the conduct of his masteral thesis from which the present study was based. Many thanks are due Mrs. C. M. Hon and Mr. C. L. Lin for their assistance in the experimental work.

References

1. Wolters, W. R., G. S. Libey and L. L. Chrisman (1982) Effect of triploidy on growth and gonad development of channel catfish. Trans. Am. Fish. Soc., **111**: 102–105.
2. Tabarini, C. L. (1984) Induced triploidy in the bay scallop, *Argopecten irradians,* and its effect on growth and gametogenesis. Aquaculture, **42**: 151–160.
3. Suzuki, R., T. Nakanishi and T. Oshiro (1985) Survival, growth and sterility of induced triploids in the cyprinid loach, *Misgurnus anguillicaudatus.* Bull. Jap. Soc. Sci. Fish., **51**(6): 889–894.
4. Krasznai, Z. and T. Marian (1986) Shock–induced triploidy and its effect on growth and gonad development of the European catfish, *Silurus glanis.* J. Fish Biol., **29**: 519–527.
5. Lincoln, R. F. and A. P. Scott (1984) Sexual maturation in triploid rainbow trout, *Salmo gairdneri* (Richardson). J. Fish Biol., **25**: 385–392.
6. Stanley, J. G., S. K. Allen, Jr. and H. Hidu (1981) Polyploidy induced in the American oyster, *Crassostrea virginica,* with cytochalasin B. Aquaculture, **23**: 1–10.
7. Valenti, R. J. (1975) Induced polyploidy in *Tilapia aurea* (Steindachner) by means of temperature shock treatment. J. Fish Biol., **7**: 519–528.
8. Chourrout, D. and J. Itskovich (1983) Three manipulations permitted by artificial insemination in tilapia: Induced diploid gynogenesis production of all triploid populations and intergeneric hybridization. In: L. Fishelson and Z. Yaron (Comps.). Proceedings: International Symposium on Tilapia in Aquaculture, 1983, The Organizing Committee, International Symposium on Tilapia in Aquaculture, Department of Zoology, George S. Wise Faculty of Life Sciences, pp. 246–255.
9. Don, J. and R. R. Avtalion (1986) The induction of triploidy in *Oreochromis aureus* by heat shock. Theor. Appl. Genet., **72**: 186–192.
10. Don, J. and R. R. Avtalion (1988) Comparative study on the induction of triploidy in tilapias, using cold- and heat-shock techniques. J. Fish Biol., **32**: 665–672.
11. Pandian, T. and K. Varadaraj (1987) Techniques for producing all-male and all-triploid *Oreochromis mossambicus.* In: R. S. V. Pullin, T. Bhukaswan, K. Tonguthai and J. L. Maclean (Eds). The Second International Symposium on Tilapia in Aquaculture. ICLARM Conf. Proc. 15, Department of Fisheries,

Banghok, Thailand, and International Center for Living Aquatic Resources Management, Manila, Philippines. pp. 243–249.

12. Swarup, H. (1959) Effect of triploidy on the body size, general organization and cellular structure in *Gasterosteus aculeatus* (L.) J. Genet., **56**: 143–155.
13. Purdom, C. E. (1972) Induced polyploidy in plaice, *Pleuronectes platessa* and its hybrid with flounder, *Platichthys flesus*. Heredity, **29**(1): 11–24.
14. Gervai, J., S. Peter, A Nagy, L. Horvath and V. Csanyi (1980) Induced trioploidy in carp, *Cyprinus carpio* L. J. Fish Biol., **17**: 667–671.
15. Utter, F. M., O. W. Johnson, G. H. Thorgaard and P. S. Rabinovitch (1983) Measurement and potential applications of induced triploidy in Pacific salmon. Aquaculture, **35**: 125–135.
16. Solar, I. I., E.M. Donaldson and G. A. Hunter (1984) Induction of triploidy in rainbow trout (*Salmo gairdneri* R.) by heat shock, and investigation of early growth. Aquaculture, **42**: 57–67
17. Stanley, J. G., H. Hidu and S. Allen (1984) Growth of American oysters increased by polyploidy induced by blocking meiosis I but not meiosis II. Aquaculture, **37**: 147–155.
18. Komaru, A. and K. T. Wada (1989) Gametogenesis and growth of induced triploid scallops, *Chlamys nobillis*. Nippon Suisan Gakkaishi, **55**(3): 447–452.
19. Chang, S. L. and I. C. Liao (1994) Triploidy induced by heat shock in *Oreochromis aureus*. Proc. In: R. S. V. Pullin, J. Lazard, M. Legendre, J. B. Amon Kothias and D. Pauly (Eds). The Third International Symposium on Tilapia in Aquaculture. ICLARM Conf. Proc. 41, International Center for Living Aquatic Resources Management, Manila, Philippines. (in press).
20. Karpinski, B. and J. P. Hallier (1988) Preliminary results on yellowfin spawning in the western Indian Ocean. FAO/IPTP/TWS/88/ **03**: 50–59.
21. Hassani, S. and B. Stequert (1990) Sexual maturity, spawning and fecundity of the yellow-fin tuna, *Thunnus albacares* of the Western Indian Ocean. FAO/IPTP/TWS/90/**68**: 91–97.
22. Hussain, M. G., D. J. Penman and B. J. McAndrew (1994) Effects of triploidy on sexual maturation and reproduction in nile tilapia, *Oreochromis niloticus* L. In: R. S. V. Pullin, J. Lazard, M. Legendre, J. B. Amon Kothias and D. Pauly (Eds). The Third International Symposium on Tilapia in Aquaculture. ICLARM Conf. Proc. 41, International Center for Living Aquatic Resources Management, Manila, Philippines. (in press).
23. 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.
24. Myers, J. M. (1986) Tetraploid induction in *Oreochromis* spp. Aquaculture, **57**: 281–287.

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(1992 年 12 月 29 日接受)

三倍體歐利亞吳郭魚的成長及性腺發育比較研究

摘 要

以熱擊誘發之三倍體及二倍體歐利亞吳郭魚，飼育在室外池之吊網中進行成長比較試驗結果，24週齡的二倍體及三倍體者間之成長沒有明顯的差別(P>0.05)。三倍體之生殖突起沒有明顯的發育，不過，其生殖腺指數則明顯的比二倍體者小(P<0.01)，三倍體的精巢組織中之精子呈現大小不一的現象，而24週齡的二倍體魚之卵細胞已經有明顯的卵黃堆積現象，相反的，絕大部份的三倍體之卵細胞仍停留在卵原細胞期。

關鍵字：歐利亞吳郭魚，二倍體，三倍體，成長，性腺發育

張賜玲, 張正芳, 廖一久 (1993) 三倍體歐利亞吳郭魚的成長及性腺發育比較研究. 水產研究, 1(1): 43–49.