Asian Fisheries Science 6(1993):213-217.
Asian Fisheries Society, Manila, Philippines
https://doi.org/10.33997/j.afs.1993.6.2.009

Induced Tetraploidy in Grass Carp (*Ctenopharyngodon idella* Val) by Heat Shock

SI-MING ZHANG, XING-ZHONG ZHANG and YONG ZENG

Yangtze River Institute of Fisheries
Chinese Academy of Fisheries Science
Shashi-434000, Hubei
China

Abstract - Tetraploid grass carp (*Ctenopharyngodon idella* Val) were produced by heat shock to the eggs before first cleavage. Heat was applied 0-20 min. before cleavage at 39-42°C for 1-4 min. The highest percentage of tetraploids (42%) was at 40°C for 2 min., 10 min. before first cleavage. The effect of temperature on embryo survival is described.

Inducing polyploidy through chromosome set manipulation in fish is of recent origin. Application of polyploidy in aquaculture generally involves triploids, as they will be used for commercial fish culture, sex or population control, etc. (Thorgaard 1986; Zhang 1990). However, inducing triploidy is a difficult process.

In rainbow trout, tetraploids crossed with diploids produced triploids (Chourrout et al. 1986). This method is simple, economical and holds promise in obtaining triploids. Thus, the major goal is to develop a method for producing tetraploids. So far tetraploids have been induced in rainbow trout (Refstie 1981; Chourrout et al. 1982; Ma et al. 1987); channel catfish (Bidwell et al. 1985); bighead carp (Aldridge et al. 1990; Hong 1990); tilapia (Valenti 1975; Don and Avtalion 1988); a hybrid of *Ctenopharyngodon idella* x *Megalobrama amblycephala* (Zhang et al. 1986); and a hybrid of white crucian carp x red carp (Chen et al. 1987).

The grass carp (*Ctenopharyngodon idella* Val) is of value as a food fish and a biological control agent for aquatic weeds (Bardach et al. 1972; Sutton 1977). Production of triploid grass carp has been reported (Su et al. 1983; Cassani and Caton 1985, 1986). However, no reports on inducing tetraploidy in grass carp are available. We

report here the induction of tetraploidy in grass carp through heat shock.

Healthy grass carp broodstock were taken from the fisheries experimental station of the Yangtze River Institute of Fisheries, Chinese Academy of Fisheries Science, and injected with LRH-a + HCG in two split doses for inducing ovulation. The first injection was followed 5-6 hours later by the second injection. After injections the brood fish were placed in a spawning tank with water circulation to stimulate ovulation. Ripe fish were collected immediately and stripped.

In each experiment, a few eggs were fertilized prior to mass fertilization, so as to evaluate the times of embryonic development of different stages. The fertilized eggs were collected in small baskets, then immersed in constant-temperature water tanks for various durations and temperatures. After the heat-shock treatment, the eggs were transferred to normal water for incubation.

The chromosome examinations were performed on embryos, from segmentation to the eye crystalline lens formation stage. About 100 embryos were chosen and transferred to a 5 ml centrifuge tube. A pipette whose opening was just bigger than the embryo was used for removing chorion. Next, the embryos were washed with 0.85% NaCl solution twice. The embryos were agitated with the pipette, so that the cells were released. After that, 4.5 ml of 0.85% NaCl solution and 0.5 ml colchicine at a concentration of 0.05% were added to each centrifuge tube and the contents allowed to settle for 50-60 min. at room temperature (24-30°C). The cells were collected by centrifuging and transferred to double distilled water for 6 min. for hypotonic treatment. After centrifugation and decanting the hypotonic solution, the cells were fixed in two changes of 3:1 methanol-acetic acid. The cell suspension was dropped onto a chilled slide, air-dried and stained in 10% Giemsa for 20 min. Usually, more than 100 chromosome metaphase spreads (including 2n and 4n) were counted in each treatment for determining the percentage of tetraploidy. Tetraploid grass carp cells should contain 96 chromosomes, but counts varied from 85-110 in this study.

The results showed that the heat shock administered at 39-42°C, for 1-4 min., 0-20 min. prior to first cleavage of eggs could induce tetraploidy (Table 1). The optimal parameters of this method were considered to be 40°C for 2 min., 10 min. prior to first cleavage. This produced 41.6% tetraploids.

Table 1. Effect of heat shocks on inducing tetraploidy in *C. idella*.

Treatment temp. (°C)	Duration (min.)	Time prior to first cleavage (min.)	No. metaphase spreads analyzed	Tetraploids (%)
39	1	20	105	3.8
39	2	20	- *	- *
39	3	20	- *	- *
39	4	20	200	23.5
40	1	10	200	10.0
40	2	10	149	41.6
40	2	20	94	22.3
40	2	30	- *	- *
40	2	40	105	3.8
40	3	20	- *	- *
40	4	10	- *	- *
41	1	-2	158	19.5
41	1	0	113	28.5
41	1	5	88	31.9
41	1	15	233	5.1
41	2	15	138	15.8
41	3	15	112	23.2
41	4	15	102	20.8
42	1	10	171	35.6
42	2	10	105	26.7
42	3	10	100	20.0
42	4	10	- *	- *

*Eggs died due to lack of oxygen. Chromosome checking was not carried out.

Besides tetraploids (4n) and diploids (2n), triploids (3n) and pentaploids (5n) were also noticed.

The effect of temperature on viability of eggs was also studied. Average survival rate mostly depended on the treatment temperature and duration. Survival of embryos and temperature or duration had a negative correlation. Usually, the embryo survival rate was above 80% relative to control at 39°C for 1-3 min. duration (Fig. 1). Temperatures of more than 43°C, for a duration of 3 min. resulted in almost 100% mortality prior to hatching.

In rainbow trout (Thorgaard et al. 1981) and channel catfish (Bidwell et al. 1985), precise timing of the postfertilization shock was not critical. But Hong (1990) found that timing of postfertilization shock was an important factor for producing high percentages of bighead carp tetraploids. Our study demonstrated that shocks given 0-15 min. prior to first egg cleavage induced high percentages of tetraploidy. We have investigated more than 20 different treatment groups and more than 100 metaphase spreads in

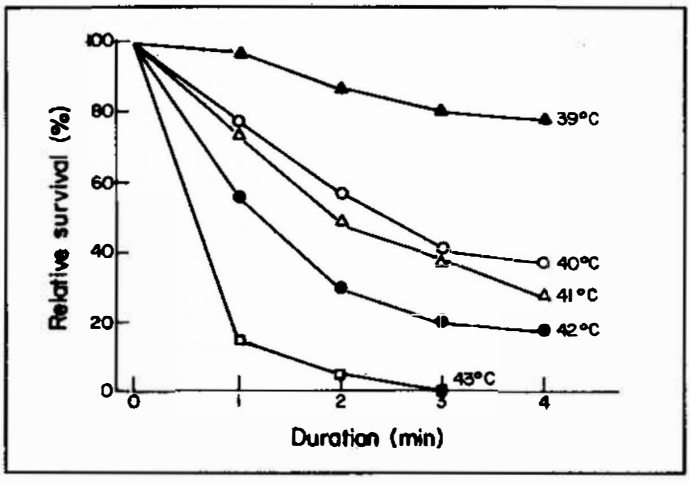

Fig. 1. Effect of heat shock on survival of embryo.

each treatment. None of the treatments gave percentages of tetraploids above 50%. This is due to nonsynchronized development of the eggs (embryos). Though some embryos have a very short sensitive stage for polyploidy, the majority of embryos have a relatively long sensitive stage, so the timing of postfertilization shocks is usually not critical.

Acknowledgements

The authors thank Dr. P.V.G.K. Reddy, senior scientist (Fish Genetics Division), Central Institute of Freshwater Aquaculture, India, for going through the manuscript and suggesting improvements; and Mrs. Hong-Mei Fang for typing the manuscript.

References

Aldridge, F.J., R.Q. Marston and J.V. Shireman. 1990. Induced triploids and tetraploids in bighead carp, *Hypophthalmichthys nobilis* verified by multi-embryo cytofluorometric analysis. Aquaculture 87:121-131.

Bardach, J.E., J.H. Ryther and W.O. Mclarney. 1972. Aquaculture: The farming and husbandry of freshwater and marine organisms. Wiley-Inter Science, New York.

Bidwell, C.A., C.L. Chrisman and G.S. Libey. 1985. Polyploidy induced by heat shock in channel catfish. Aquaculture 51:25-32.

Cassani, J.R. and W.E. Caton. 1985. Induced triploidy in grass carp, *Ctenopharyngodon idella* Val. Aquaculture 46:37-44.

Cassani, J.R. and W.E. Caton. 1986. Effect production of triploid grass carp (*Ctenopharyngodon idella*) utilizing hydrostatic pressure. Aquaculture 55:43-50.

Chen, M.R., K. Yan, H.Q. Lin, Y.L. Yi, X.Q. Yang, P.L. Liu and H.X. Chen, 1987. Preliminary study on artificially induced heterogeneous tetraploid in the hybrid of white crucian carp x red carp. Acta Hydrobiol. Sin. 11:96-98. (In Chinese.)

Chourrout, D. 1982. Tetraploidy induced by heat stock in rainbow trout *Salmo gairdneri* R. Reprod. Nutr. Develop. 22:569-574.

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.

Don, J. and R.R. Avatalion. 1988. Production of viable tetraploid tilapia using the cold shock technique. Bamidgeh 40(1):17-21.

Hong, Y.H. 1990. Tetraploid induced by heat shock in bighead carp, *Aristichthys nobilis*. Acta Zool. Sin. 36:71-80. (In Chinese with English abstract.)

Ma, T., C.B. Zhu and B.R. Zhu. 1987. Tetraploid of rainbow trout (*Salmo gairdneri* Richardson) induced by heat shocks. Acta Hydrobiol. Sin. 11:336-342. (In Chinese with English abstract.)

Refstie, T. 1981. Tetraploid rainbow trout produced by Cytochalasin B. Aquaculture 25:51-58.

Su, Z.G., K.S. Xu and G.D. Bai. 1983. Studies on triploid grass carp and its karyotype. Trans. Chinese. Ichthyol. Soc. 3:53-59. (In Chinese with English abstract.)

Sutton, D.L. 1977. Grass carp (*Ctenopharyngodon idella* Val) in North America. Aquat. Bot. 3:157-164.

Thorgaard, G.H., M.E. Jazwin and A.R. Stier. 1991. Polyploidy induced by heat shock in rainbow trout. Trans. Am. Fish. Soc. 110:546-550.

Thorgaard, G.H. 1986. Ploidy manipulation and performance. Aquaculture 57:57-64.

Valenti, R.J. 1975. Induced polyploidy in *Tilapia aurea* (Steindachner) by means of temperature shock treatment. J. Fish Biol. 7:517-528.

Zhang, X.Z., Y.H. Hong, S.-M. Zhang and K.H. Fong. 1986. Heat shock induced allotetraploid of *Ctenopharyngodon idella* x *Megalobrama amblycephala*, p. 132-133. *In* The First All-China Symposium on Agricultural Biotechnology. (Abstract in Chinese.)

Zhang, S.-M. 1990. Genome manipulation in fish and perspective of its application. Chinese. J. Zool. 25: 41-45. (In Chinese.)

Manuscript received 13 May 1991; accepted 26 February 1992.