Triploidy Induction in Stinging Catfish *Heteropneustes fossilis* Using Cold Shock

A.A. GHEYAS[1], M.F.A. MOLLAH[1] and M.G. HUSSAIN[2]

[1]Department of Fisheries Biology and Genetics
Bangladesh Agricultural University
Mymensingh-2202
Bangladesh

[2] Bangladesh Fisheries Research Institute
Mymensingh -2202
Bangladesh

Abstract

A study was conducted to induce triploidy in newly fertilized eggs of *H. fossilis* using cold shock. The eggs were exposed at two temperature-regimes viz. 2^0C and 4^0C. Among the various combinations of temperature and duration tested, shock duration for 10 min at 2^0C applied 3 min after fertilization was the best; it induced triploidy up to 94 to 97% of the eggs and had the best hatching and survival percentage of the triploid larvae. The success of triploidy induction was determined by chromosome counting. Erythrocyte measurements from diploid and triploid individuals were compared to results of karyotyping which showed a significant increase ($P < 0.05$) in the erythrocyte nuclear major and minor axes, area and volume in triploids over those in diploids. The implications of the results for production of genetically sterile triploidy in stinging catfish are briefly discussed.

Introduction

As a technique of chromosome engineering, triploidy induction has earned special interest in producing sterile populations of fish. Sexual development of fish is thought to reduce the somatic growth as a major part of the nutrient and energy is used for sexual maturation (Purdom 1976, Utter et al. 1983). Sterilization can overcome the detrimental effects of sexual maturation on normal growth of fish. Sterility in fish can be induced either by exogenous hormone treatment (Hunter and Donaldson 1983) or by triploidy induction (Thorgaard 1983, Hussain et al. 1991, Hussain 1996). Since hormone treatment often leads to consumer rejection, triploidy induction offers the most promising mechanism for sterility induction. Sterility in triploid fish occurs due to failure of homologous chromosomes to synapse correctly during the first meiotic division (Hussain et al. 1991). Triploidization for sterility induction has been applied in species which attain sexual maturity before reaching marketable size, die after spawning or are prolific breeders that reduced their somatic growth (Hussain et al. 1991, Malison et

al. 1993). Triploidy can be produced by either of the two mechanisms. The first is to subject newly fertilized eggs to thermal (either heat or cold) or pressure shock in order to prevent the extrusion of second polar body during the second meiotic division of the eggs. This incorporates the one set of chromosomes of the polar body in the zygote cell, thereby making it triploid. The second mechanism is to produce tetraploids and then using them as broodstock to cross with normal diploid to produce triploid offspring.

Stinging catfish *H. fossilis* of family Heteropneustidae is an indigenous species of Indian subcontinent, which shows antagonistic relationship between its reproductive process and bodily growth. The fish attains sexual maturity in about one year (Khan 1972) and has a long breeding season and spawns several times at short intervals during each breeding cycle (Sundararaj 1959). These characteristics slow somatic growth and extend the time to reach marketable size which for this fish is about one and half years. To overcome this problem, triploidy induction to produce genetically sterile stock has been identified as a potential approach towards shortening the culture period. In this study, we attempted to induce triploidy in stinging catfish using cold shock.

Materials and Methods

Collection of brood fish, procurement of eggs and sperms

The study was conducted at the Freshwater Station of Bangladesh Fisheries Research Institute, Mymensingh, Bangladesh. The brood fish were taken from the pond complex of the Institute. For collection of eggs, only the female spawners were injected with carp pituitary gland (PG) at the rate of 70 mg·kg^{-1} body weight. The females ovulated within 8 to 10 hours of PG injection and the eggs were collected by stripping. Milt cannot be stripped, so testes were removed from mature males and were macerated in 0.85% sodium chloride solution. The eggs and milt were mixed thoroughly using a soft feather to enable fertilization and a small amount of water was added to activate the spermatozoa. This method of induced breeding for this fish has been developed at BFRI and is routinely followed there.

Application of cold shock

Cold shock of activated eggs was applied in a thermostatically controlled water bath (Model FDPGH, Techne Cambridge, Ltd, UK). Immediately after fertilization, eggs (ca 100) were taken in small strainers that were then placed in the water bath. Two cold shocks were tested. In the first series, the temperature shock was at 2^{0}C and the duration of shock application varied from 5 to 35 min with an increment of 5 min between successive steps. In the second series of work, shock temperature was maintained at 4^{0}C and the duration varied between 10 to 40 min again with an increment of 5 min. In both series, the time of initiation of shock treatment was 3 min after activation. During each trial, one batch of eggs was not shocked and served as control.

Parameters studied

The optimum combination of temperature and duration for triploidy induction was determined considering three factors: the highest rate of triploidy induction, the highest hatching rate and the maximum survival percentage from hatching to yolk sac absorption stage. The data on hatching and survival rates of the cold-shock groups are presented as the percentage relative to the control group. The rate of triploidy induction was assessed through chromosome counting. A study was also conducted using RBC nuclear size to compare the result of chromosome counting.

Karyological analysis

The method described by Hussain and McAndrew (1994) was followed for chromosome karyotyping. The tissues for this purpose were collected from newly hatched or 1-day-old larvae of the control and treated groups. The metaphase spreads of chromosomes were examined and the chromosome numbers scored by examining the slides under X400 (oil immersion) magnification. Chromosome counting was done from several spreads (more than 5) per slide and from 10 to 15 individuals from each group.

Erythrocyte measurement

A comparative study on various size parameters of the erythrocytes and their nuclei from normal diploid fish and putative triploid was conducted to observe the effect of triploidization on their respective sizes. Offsprings of the control and putative triploid groups were reared for about two months to enable them to become large enough for blood collection. Blood samples were collected from seven triploid and seven control group fingerlings. Smears were produced on glass slides and were fixed for two minutes in absolute methanol. The smears were subsequently stained with Wright's blood stain for 10 to 15 min, washed in distilled water, air dried and finally mounted in DPX (a mixture of Distrene: Dibutyl phthalate: Xylol). Major axes (a) and minor axes (b) of erythrocyte cells and their nuclei were measured for 25 cells from each fish with an eye piece glass micrometer under 400 times magnification. Areas and volumes of the cells and their nuclei were computed using the following formulae given by Krasznai et al. (1984b).

$$\text{Surface area } S = \frac{a.b.\pi}{4}$$

$$\text{Volume } V = \frac{4}{3}\pi\left(\frac{a}{2}\right)\left(\frac{b}{2}\right)^2$$

Where
a = mean values of major axes
b = mean values of minor axes

Statistical analysis

The results of the various trials were statistically compared using a Test of Homogeneity of Variances before ANOVA followed by Duncan's Multiple Range Test (DMRT). These statistical analyses were performed with the aid of the computer software Mstat-C program (designed by the Crop and Soil Science Department, Michigan State University). Test of significance of erythrocyte measurements was done using t-test.

Result

Table 1 and figures 1 to 3 present the results on the application of cold shock at two temperature regimes to induce triploidy. Table 1 and figure 3 indicate that of the two temperatures tested 2^0C gave the highest percentage of triploid (ranging 95 to 97%) when the shock was applied three minutes after fertilization for 10 min and more. From 10 min onward no significant difference ($P < 0.05$) was observed in the rate of triploidy induction. In contrast, the triploidy rate at 4^0C was considerably lower, the highest level achieved being only about 70% as against 97% at 2^0C (Fig. 3). Statistical analysis indicates that at 4^0C the duration of 20 min and above was equally efficient in inducing triploidy.

Table 1. Effect of temperature and cold shock duration on various parameters of triploidy induction in *H. fossilis.* The time of initiation of cold shock in each trial was 3 min.

Shock temp	Duration of shock treatment (min)	Fertilization rate (%)	Hatching rate (%RC)	Survival of hatchlings up to yolk sac resorption (% RC)	Triploidy rate (%)
2^0C	5	$95.54 \pm 2.90\ a_1$	$47.40 \pm 7.31\ a_1$	$49.45 \pm 9.34\ b_1$	$44.89 \pm 4.31\ b_1$
	10	$93.89 \pm 6.07\ a_1$	$57.32 \pm 13.92\ a_1$	$71.14 \pm 9.70\ a_1$	$95.14 \pm 1.46\ a_1$
	15	$93.02 \pm 6.02\ a_1$	$50.89 \pm 6.20\ a_1$	$33.15 \pm 7.53\ c_1$	$95.63 \pm 1.81\ a_1$
	20	$91.48 \pm 1.84\ a_1$	$26.44 \pm 19.39\ b_1$	$19.31 \pm 9.16\ d_1$	$96.40 \pm 1.12\ a_1$
	25	$90.79 \pm 3.66\ a_1$	$2.08 \pm 3.00\ c_1$	$0 \qquad\qquad e_1$	$96.77 \pm. 0.79\ a_1$
	30	$89.33 \pm 3.73\ a_1$	$0 \qquad\qquad c_1$	$0 \qquad\qquad e_1$	–
	35	$83.30 \pm 5.61\ b_1$	$0 \qquad\qquad c_1$	$0 \qquad\qquad e_1$	–
4^0C	10	$93.82 \pm 3.19\ a$	$40.19 \pm 20.39\ a$	$8.90 \pm 2.11\ bcd$	$0 \qquad\qquad c$
	15	$92.63 \pm 5.20\ a$	$35.22 \pm 18.23\ ab$	$22.87 \pm 6.34\ bc$	$20.06 \pm 2.52\ b$
	20	$88.88 \pm 7.23\ a$	$41.56 \pm 14.13\ a$	$57.78 \pm 6.50\ a$	$55.43 \pm 11.22a$
	25	$88.68 \pm 2.85\ a$	$24.80 \pm 6.16\ abc$	$68.31 \pm 15.5\ a$	$67.27 \pm 2.25\ a$
	30	$89.69 \pm 7.95\ a$	$16.83 \pm 2.69\ bc$	$27.62 \pm 11.68\ b$	$64.30 \pm 7.18\ a$
	35	$86.00 \pm 7.19\ a$	$10.33 \pm 6.63\ c$	$4.26 \pm 2.67\ cd$	$67.68 \pm 2.23\ a$
	40	$86.00 \pm 10.02\ a$	$4.84 \pm 5.85\ c$	$0.44 \pm 0.76\ c$	$69.83 \pm 1.16\ a$
[2]Control		98.33 ± 1.65	48.41 ± 13.68	80.28 ± 2.71	
[4]Control		93.33 ± 5.29	41.04 ± 15.60	74.59 ± 8.20	

* % RC represents percentage relative to control where the values have been calculated by adjusting the control to 100%.The values of the control represent actual data.

The number of replication for each duration at 2^0C was 5 and at 4^0C was 3.

The letters a_1 to e_1 and a to e indicate statistical comparison. Values bearing same notations are statistically insignificant.

Although longer shock durations at both 2^0C and 4^0C increased the rates of triploidy induction, they generally had an adverse effect on hatching and survival performances. Figure 1 shows that hatching rate at 2^0C decreased gradually after 10 min of shock duration. However statistical analysis reveals that the hatching performances at 10 and 15 min were not significantly different. Actual significant decrease in hatching was observed after durations of 20 min or more. The lower hatching rate below 10 min duration was due to preponderance of aneuploid and mosaic embryos as reported under karyological examination latter in this paper. Similarly at 4^0C the highest hatching was achieved at 20 min duration and the rate decreased gradually with longer durations (Fig 1).

Figure 2 shows that hatchlings produced at cold shock of 10 min at 2^0C had the highest survival rate. Both below and above this duration the survival was lower. The highest survival rate at 4^0C was observed at cold shock of 25 min. Statistically, however the effect of the cold shock durations of 20 and 25 min at 4^0C was not significantly different.

Thus considering all the indices together viz, hatching, survival and triploidy induction rates at 2^0C, 10 min cold shocking was found to be the best; whereas at 4^0C, 20 or 25 min were the most effective.

Hatching and survival rates of cold shock treated groups were lower than those of the control group. In order to facilitate comparison the hatching and survival rates of cold shock groups were expressed in terms of percent relative to control (%RC) and for the control group actual rates were presented in table 1.

Ploidy level by cold shock was assessed by karyological analysis. In normal diploid *H. fossilis* the number of chromosome was 2n = 56 and in triploid chromosome number was 3n = 84. All suboptimal cold shocking featured a preponderance of mosaics as assessed by karyological examination. These mosaic individuals in most cases showed distinct morphological deformities and did not survive more than three to four days.

The comparative data on the erythrocyte nuclear size is presented in table 2. The increase in chromosome numbers in triploid groups increased the erythrocyte measurements (P < 0.05) over those in diploids. The ratios of all the parameters of triploid erythrocyte to those of diploids were greater than unity indicating strongly that triploid red blood cells (RBC) were larger than the diploid ones. The frequency distribution of the mean
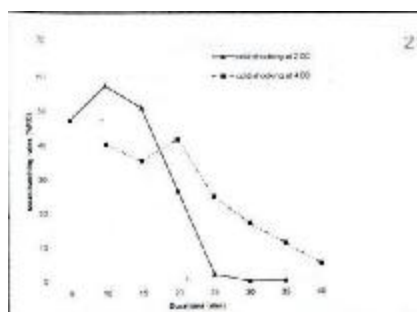

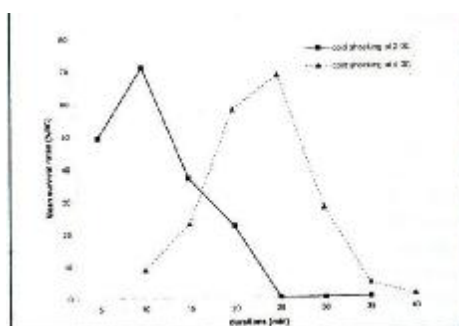Fig. 1. Effect of cold shock on hatching percentage of *H. fossilis* embryo.

Fig. 2. Effect of cold shock on the eggs and subsequent survival rate of *H. fossilis* hatchlings.
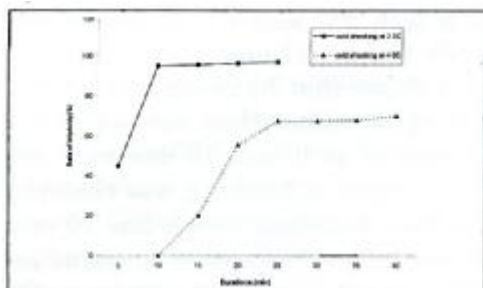
328

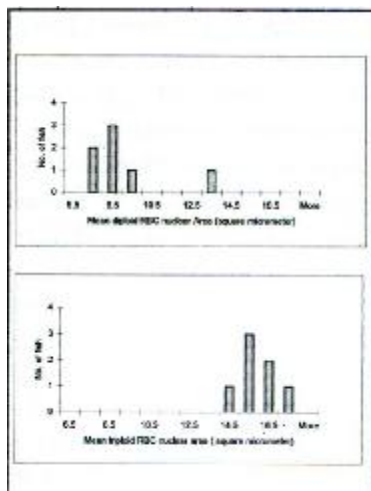Fig. 3. Effect of cold shock on triploidy induction rate of *H. fossilis*.

Fig. 4. Frequency distribution of mean erythrocyte nuclear area of diploid control and putative triploid *H. fossilis*.
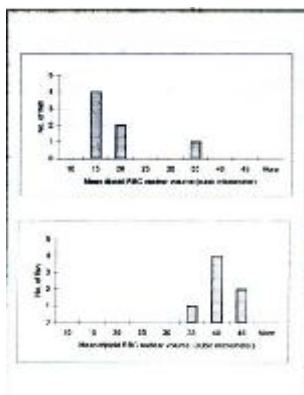

Fig. 5. Frequency distribution of mean erythrocyte nuclear volume of diploid control and putative triploid *H. fossilis*.
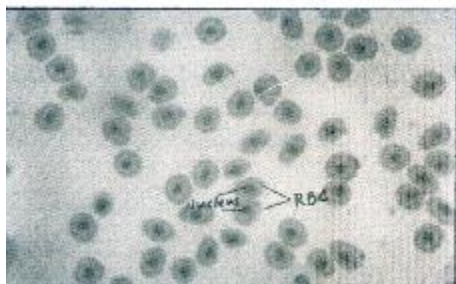

Fig. 6. Erythrocytes of triploid *H. fossilis* blood showing normal size of the cell nucleus (x1000 magnification)
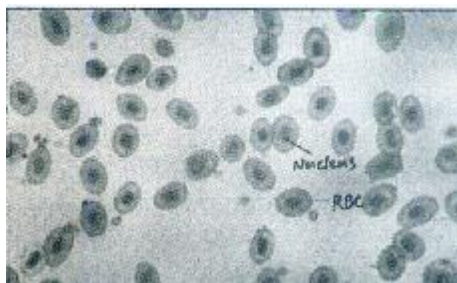

Fig. 7. Erythrocytes of triploid *H. fossilis* blood showing nucleus larger than that of diploid cells (x1000 magnification)

Table 2. Comparison of erythrocyte and their nuclear sizes between normal diploid and triploid *H. fossilis* (major and minor axes in micrometer, area in sq. micrometer, volume in cubic micrometer).

Parameters	Normal diploid (n = 7 fish)			Triploid (n = 7 fish)			Ratio of triploid to normal diploid
Erythrocyte							
Major axes (a)	10.244	±	0.311	12.57	±	0.671	1.227
Minor Axes (b)	8.359	±	0.363	9.413	±	0.983	1.126
Area (S)	67.304	±	4.592	93.249	±	14.551	1.090
Volume (V)	375.979	±	43.045	593.230	±	156.804	1.578
Nuclei							
Major axes (a)	3.630	±	0.426	5.329	±	0.188	1.468
Minor Axes (b)	2.939	±	0.320	3.72	±	0.132	1.266
Area (S)	8.469	±	2.023	15.719	±	0.952	1.856
Volume (V)	16.959	±	6.369	38.688	±	3.558	2.281

values of erythrocyte nuclear areas and volumes for diploid control and triploid had little overlap (Figs. 4 and 5). On the contrary, the area and volume of triploid individuals showed a propensity to reside at the upper range than diploid erythrocytes. Figures 6 and 7 represent the erythrocytes of diploid and triploid individuals respectively. The plates clearly show that the nuclei of the triploid erythrocytes are considerably larger than those of the diploids.

Discussion

The present experiment was successful in the induction of triploidy in stinging catfish achieving about 95 to 97% triploidy using cold shock three minutes after fertilization at a temperature of 2^0C with a duration of 10 min or upward. Hence, cold shock is an effective means for chromosome manipulation in this species. It is generally thought that warm water species are more susceptible to cold shock (Wolter et al.1981) than to heat shock, whereas heat shock is more effective for cold water species (Chourrout 1980 and Nagy 1987). The present experiment verified this contention as stinging catfish is an inhabitant of warm climate and is highly susceptible to cold shock treatments.

The occurrence of mosaic offsprings (having different ploidy levels in the cells of the same individual) is a frequent observation in studies regarding triploidy induction (Hussain 1995, Lemoine and Smith 1980, Stanley 1978). It is most probably the result of inadvertent exposure of the eggs to shock temperature after fertilization. In our experiment all suboptimal shock showed diploid/ triploid mosaicism among the embryos of treated groups.

The hatching and survival percentages in cold shock treated groups were considerably lower than those of control group in the present experiment. Such lower hatchability and survivability of triploid individuals compared to control have been reported by other authors as well (Chrisman et al. 1983, Krasznai et al. 1984a, Solar et al. 1984). Moreover, the hatching, survival and triploidy induction rates varied considerably among different lots of eggs subjected to the same cold shock. Similar results have also been reported in other species and have been attributed to factors such as egg quality differences or the susceptibility of eggs from different origins to shock treatments (Lou and Purdom 1984, Johnstone 1985, Shelton et al. 1986, Ezaz et al.1998). Malison et al. (1993), however, have suggested the wide range of ambient water temperature from which brood fish were captured as the probable cause for such variation in their experimental fish, perch (*Perca flavescens*). In the case of *H. fossilis* such factors in addition to egg quality could have been important. Moreover, the variation in the water temperature during the incubation period under ambient conditions might have also contributed to such differing hatching and survival rates.

Erythrocyte measurement has been used by many workers to determine the ploidy level in fish as with increase in chromosome number, the size of the erythrocytes particularly that of their nuclei increases (Swarup

1959, Cherfas 1966, Wolters et al. 1982, Krasznai et al. 1984b, Lemoine and Smith 1980, Sezaki et al. 1985, Don and Avtalion 1988, Humayun et al.1994). Different workers have suggested different variable measures of erythrocytes and their nuclei to be important in ploidy determination. According to Wolters et al. (1982) mean major axis was the best single variable in channel catfish. Penman et al. (1987) also found the same variable useful in distinguishing triploid and diploid fish. The majority of other workers, however, have given emphasis on the nuclear volume. Triploidization generally increases the nuclear volume by 1.5 times (Swarup 1959, Beck and Biggers 1983, Purdom 1972). In the case of stinging catfish, not only the nuclei were larger in triploids but also the erythrocytes themselves. Nuclear volume, however, showed a 2.28 times increase in triploids instead of the usual 1.5 times in other species. Significantly greater values ($P < 0.05$) of all variables viz. major axis, minor axis, area and volumes in triploid stinging catfish indicated that any of these parameters could be used for identification of ploidy in this species.

Conclusion

The experiment reported in this paper shows that triploidy can be successfully induced in stinging catfish through the use of cold shock at proper conditions. This work can serve as a basis for further work to test other shock protocols and to scale up the most practical method for a commercial production of triploid stock. Besides, attempts should be made to produce tetraploid and then to produce a hybrid triploid from tetraploid x diploid crosses. In this way, the adverse effects of shock application might be averted. The authors strongly believe that production of genetically sterile stinging catfish using the aforementioned techniques as in salmonids, tilapia and other fish would be of benefit to aquaculture.

Acknowledgment

References

Beck, M.L. and C.J. Biggers. 1983. Erythrocyte measurements of diploid and triploid *Ctenopharyngodon idella* x *Hypophthalmichthys nobilis* hybrids. Journal of Fish Biology 22: 497-502.

Cherfas, N.B. 1966. Natural triploidy in females of the goldfish (*Carassius auratus gibelio*). Genetica 12:16-24.

Chourrout, D. 1980. Thermal induction of diploid gynogenesis and triploidy in the eggs of the rainbow trout (*Salmo gairdneri* Richardson). Reproduction Nutrition Development 20: 727-733.

Chrisman, C.L., W.R. Wolters and G.S. Libey. 1983. Triploidy in channel catfish. Journal of the World Mariculture Society 14: 279-293.

Don, J. and R.R. Avtalion, 1988. Ploidy and gynogenesis in tilapias. *Les Colloques de I'INRA*, 44: 199-205.

Ezaz, M.T., A.T.A. Ahmed, M.S. Islam, S. Sayeed and M.G. Hussain. 1998. Triploidy induction in hybrid catfish (*Clarias batrachus* Linnaeus x *Clarias gariepinus* Burchell) using heat shock. Bangladesh Journal of Zoology 26(1): 85-94.

Humayun, N.M., D.J. Penman, M.G. Hussain and M.G.M. Alam. 1994. Erythrocyte nuclear measurements of diploid controls and heat shocked triploid tilapia *Oreochromis niloticus* (L.). Bangladesh Journal of Zoology 22(1): 17-23.

Hunter, G.A. and E.M. Donaldson. 1983. Hormonal sex control and its application to fish culture. In: W.S. Hoar, D.J. Randall and E.M. Donaldson (Editors). Fish Physiology. Vol. IX, Part B. Academic Press, New York. NY. pp. 223-303.

Hussain, M.G., A. Chatterji, B.J. McAndrew and R. Johnstone. 1991. Triploidy induction in Nile tilapia, *Oreochromis niloticus* L. using pressure, heat and cold shocks. Theoretical and Applied Genetics 81: 6-12.

Hussain, M.G. 1995. Suppression of meiotic and mitotic cell divisions in Nile tilapia, *Oreochromis niloticus* L., and induction of triploids and two types of gynogenetic diploids. Asian Fisheries Science 8: 133-142.

Hussain, M.G. 1996. Advances in chromosome engineering research in fish: Review of methods, achievement and applications. Asian Fisheries Science 9: 45-60.

Hussain, M.G. and B.J. McAndrew. 1994. An improved technique for chromosome karyotyping from embryonic and soft tissues of tilapia and salmonids. Asian Fisheries Science. 7: 187-190.

Johnstone, R. 1985. Induction of triploidy in Atlantic salmon by heat shock. Aquaculture 49:133-139.

Khan, H.A. 1972. Induced breeding of air-breathing fishes. Indian Farming 22(4): 44-45.

Krasznai, Z., T. Marian and G. Kovacs. 1984a. Production of triploid European Catfish (*Silurus glanis* L.) by cold shock. Aquacultura Hungarica 4: 25-32.

Krasznai, Z., T. Marian, Z. Jeney, G. Jeney and A. Zsigri. 1984b. Effect of triploidy on the blood cell size of hybrid grass carp. Aquacultura Hungarica (Szarvas), 4: 17-24.

Lemoine, H.L., Jr. and L.T. Smith. 1980. Polyploidy induced in Brook trout by cold shock. Transactions of the American Fisheries Society 109: 626-631.

Lou, Y.D. and C.E. Purdom. 1984. Polyploidy induced by hydrostatic pressure in rainbow trout, *Salmo gairdneri.* R. Journal of Fish Biology 25: 345-351.

Malison, J.A., T.B. Kayes, J.A. Held, T.P. Barry and C.H. Amundson. 1993. Manipulation in ploidy in yellow perch (*Perca flavescens)* by heat shock, hydrostatic pressure shock, and spermatozoa inactivation. Aquaculture 110: 229-242.

Nagy, A. 1987. Genetic manipulation performed on warm water fish. *In:* Proceedings of World Symposium on Selection, Hybridization and Genetic Engineering in Aquaculture, Scriften der Bundesforschungsanstalt fur Fischeri: 127-145.

Penman, D.J., D.O.F. Skibinsik and J.A. Beardmore. 1987. Survival, growth and maturity in triploid tilapia. *In* Proceedings of World Symposium on Selection, Hybridization and Genetic Engineering in Aquaculture II: 239-253

Purdom, C.E. 1972. Induced polyploidy in plaice (*Pleuronectes platessa*) and its hybrid with flounder (*Platichthys flesus*) Heredity 29: 11-24

Purdom, C.E. 1976. Genetic techniques in flatfish culture. Journal of the Fisheries Research Board of Canada. 33: 1088-1093.

Sezaki, K., H. Kobayashi, S. Watanabe and K. Hashimoto. 1985. Erythrocyte size and polyploidy of cobitid fishes in Japan. Bulletin of the Japanese Society of Scientific Fisheries 51: 777-781.

Shelton, C.J., A.G. MacDonald and R. Johnstone. 1986. Induction of triploidy in rainbow trout using nitrous oxide. Aquaculture 58: 155-159.

Solar, I.I., E.M. Donaldson and G.A. Hunter. 1984. Induction of triploidy in rainbow trout (*Salmo gairdneri* Richardson) by heat shock and investigation of early growth. Aquaculture 42: 57-67.

Stanley, J.G. 1978. Reproductive sterility in polyploid brook trout, *Salvelinus fontinalis.* Transactions of the American Fisheries Society 107: 473-478

Sundararaj, B.I. 1959. A study of correlation between the structure of the pituitary gland of the Indian catfish *Heteropneustes fossilis* and the seasonal changes in the ovary. Acta Anatomica 37: 47-80.

Swarup, H. 1959. Effect of triploidy on the body size, general organisation and cellular structure in *Gasterosteus aculeatus*(L). Journal of Genetics 56:143-155.

Thorgaard, G.H. 1983. Chromosome set manipulation and sex control in fish. In: W.S. Hoar, D.J. Randall and E.M. Donaldson (Editors). Fish Physiology. Vol. IX, Part B. Academic press New York. NY, pp. 405-434.

Utter, F.M., O.W. Johnson, G.M. Thorgaard, and P.S. Rabinovitch. 1983. Measurement and potential applications of induced triploidy in Pacific Salmon. Aquaculture 35: 125-135.

Wolters, W.R., G.S. Libey and C.L. Chrisman. 1981. Induction of triploidy in channel catfish. Transactions of the American Fisheries Society 110: 310-312.

Wolters, W.R., G.S. Libey and C.L. Chrisman. 1982. Effect of triploidy on growth and gonad development of channel catfish. Transactions of the American Fisheries Society 111:102-105.