Caspian J. Env. Sci. 2012, Vol. 10 No.2 pp. 195~204
©Copyright by University of Guilan, Printed in I.R. Iran

CJES
Caspian Journal of Environmental Sciences

[Research]

Induction of triploidy in grass carp *Ctenopharyngodon idella* Valenciennes, 1844: Comparison of cold & heat shocks

M. Hassanzadeh Saber*, M. Pourkazemi

Gene Bank Center, International Sturgeon Research Institute, P.O. Box: 41635-3464 Rasht, Iran.
** Corresponding author's E-mail: saber_meraj@yahoo.com*

(Received: Sept. 01-2011, Accepted: Feb. 20-2012)

ABSTRACT

Triploidy in grass carp, *Ctenopharyngodon idella* Valenciennes, 1844, was induced on fertilized eggs to compare cold and heat shocks. Two simplified methods explained for verification of triploidy in grass carp. The cold shock (7 ˚C) was given in three treatments for 30 min starting 2.0, 2.5 and 4.0 min after fertilization. In cold shock, the start point (2.0 min after fertilization) showed the highest rate of triploidy (60.9%). Heat shocks were given at 38 ˚C, 40 ˚C and 42 ˚C, at 4.0 min after fertilization and lasted for 1.0 min. Produced larvae using heat shock 38 ˚C showed 10.8% triploidy, but no signs of triploidy were seen in other heat shock treatments. Verification of triploidy in grass carp was carried out using karyotyping and measurment of erythrocytes surface area and volume in fingerlings. Ratio of erythrocytes dimention and the size of their nuclei in triploids to diploids was 2.35 and 1.80, respectively. Comparison of results obtained from the application of cold and heat shocks indicated that cold shocks are more effective than heat shocks in the induction of triploidy in grass carp.

Keywords: *cold shocks; heat shocks; grass carp; triploidy.*

INTRODUCTION

Grass carp, a widely introduced species, is of value as a food fish and as a biological control agent for aquatic weeds that has recently received considerable attention in many parts of the world (Sutton, 1977; Mitzner, 1978; Opuszynski & Shireman, 1995; Mitchell & Kelly, 2006). In Iran, the grass carp was first introduced from the Soviet Union for the biological control of aquatic plants in the Anzali lagoon in 1976. Following this, grass carp breeders were introduced from Romania by the Sefidroud Company, and its propagation and cultivation started in the Sefidroud Company and other hatcheries. However the main problem lies in the undesirable and uncontrolled reproduction of this fish. The introduction of triploid grass carp has been often used to avoid this unwanted reproduction (Stanley et al. 1978; Allen & Wattendorf, 1987; Zimpfer et al. 1987).

Initial attempts to prevent unwanted naturalization of grass carp focused on producing all female populations through gynogenesis (Stanley, 1976) and hormonal implants followed by mating XX males to normal females (Boney, 1984). However monosex populations are still fertile and the process of sexing juveniles is tedious and time consuming. Clippinger and Osborne (1984) attempted surgical gonadectomies but failed due to the rapid regeneration of gonads in grass carp. Studies then focused on creating triploid hybrids through grass carp x bighead carp (*Hypophthalmichthys nobilis*) intergeneric crossing (Buck, 1979; Marian & Krasznai, 1978) but the low spontaneous production of triploid hybrids and the failure of the cross to survive led directly to the current focus on the production of pure, unhybridized triploid grass carp.

Induced polyploidy for the purpose of sterilization has become increasingly popular with aquaculturists since the first trials of the technology in the 1970's (Purdom, 1972). The process involves inhibiting the second maturation division of meiosis in the fertilized egg thereby causing retention of the extra chromosome set contained in the second polar body of the ovum (Allen & Wattendorf, 1987) by heat shock (40 ˚C, 4.75 min after

fertilization for 1 min and cold shock at 5-7˚C, 2-4 min after fertilization for 25-30 min) and pressure shock (7000-8000 PSI, 4 min after fertilization for 1-2 min) to the eggs (Cassani & Caton, 1985 and 1986). Thompson et al. (1987) also produce triploid grass carp using heat shocks (38 ˚C, 1 min after fertilization for 3.5 min). This causes the triploid condition in grass carp giving them a chromosome number of 72 (3N) instead of the natural 48 (2N) and makes them functionally sterile. The presence of these extra chromosomes in the nuclei requires a corresponding increase in nuclear size which allows for the efficient separation of diploids and triploids (Allen & Wattendorf, 1987). The present study was carried out to compare cold & heat shocks for the induction of triploidy in grass carp and triploids verification using measurement of erythrocytes dimensions and numbers of chromosomes.

MATERIALS AND METHODS

Experimental design and sexual maturation:

The study was conducted at the Shahid Ansari Bony Fishes Stock Rehabilitation and Breeding Center, Rasht, Iran. In this study, six females (2.5-4.0 kg in weight) and six males (2.0-3.1 kg in weight) of grass carp were selected. Females were given carp pituitary (2mg/kg body weight) plus luteinizing hormone releasing hormone (LHRH) (25mg/kg body weight) in two stages. The first injection was 10% of the consistent solution. The second injection was administered 12 h after the first injection. Males were injected with a pituitary dose of 2 mg/kg carp body weight in one stage that coincided with the second stage of injection of females. At the time of injection, water temperature was 20±2 ˚C. Fish ovulated 9-11 h after the second injection (Rottmann *et al.* 1991).

Sperm and Ova treatment:

Eggs and sperms were collected from breeders after anesthesia using clove flower (CF_{339}). In the present study 10±0.1 g eggs (780 eggs per g) and 2 ml sperms were used in each trial. Fertilization was done following the dry method and after about 1 min water was added to the egg and sperm mix for activation of sperms and their penetration into eggs (Rothbard, 1981).

Shock temperature was conducted by methods of Cassani and Caton (1985) with some modification. In the case of cold shocks, water temperature of 7 ˚C was used. Ambient water temperature was adjusted at 20±1 ˚C. The starting time of shock treatment differed from 2 min (Group A), to 2.5 min (Group B) and 4 min (Group C) after egg-sperm activation and lasted 30 min.

Heat shocks were given at 38 ˚C (Group D), 40 ˚C (Group E) and 42 ˚C (Group F). In all treatments heat shocks were given 4.0 min after fertilization for 1.0 min. All treatments (cold and heat shocks) were run with three replicates. A control (without shock) was run for each group using the same ambient water temperature (normally) used for fertilization.

Fertilized eggs were washed after shock treatment with tap water then transferred to Veisa incubators (7-10 L). Emergence of larvae depended on temperature of the medium and started after about 48 h (20±1˚C).

Genetic trials of triploid fish:

To identify triploid fish, chromosome spreads were prepared following the squashing method from gill, kidney or fin tissues (Kligerman & Bloom, 1977) and blood smears (Krasznai et al. 1984) were taken from fingerlings 30 days after hatch. For this purpose, grass carp longer than 70 mm in total length were consistently kept alive after bleeding. Fish were anesthetized with 10-20 mg/l quinaldine, they were bled either by hyperextending the head (tilting it back) and inserting a blood lancet into the tuberosity that rose over the juncture of the gill arches at the isthmus or by inserting the lancet under a lateral line scale at the caudal peduncle. About 5 µl of blood from the pierced branchial or caudal artery was then sampled with a micropipettor.

The dimensions of erythrocytes and their nuclei were measured using a microscope equipped with Biocom Visiol software 2000 and surface area and volume of blood cells was calculated using the following formula:

Surface area= $a.b.\pi/4$ (a=large diameter, b=small diameter) (Sezaki & Kobayasi, 1978)

Volume = $4 / 3 .\pi [a/2].[b/2]^2$ (Lemoine & Smith, 1980)

Data analysis:

Results obtained from fertilization, survival and triploidy rate (mean ± SD) were statistically analyzed using Tukey's test (95% RC) and statgraphics software 2003. Curves were plotted using Excel software 2003.

RESULTS

Triploidy in grass carp was identified using both chromosome spread and measurement of erythrocyte volume. Comparison of fertilization, hatching and triploidy rate in cold shock groups and heat shock groups in grass carp revealed the following results: in cold shocks, Group A showed 60.9±3.6 % triploidy. Fertilization rate in replicates of this group was less than 3.0% (230/7800) as compared to control showed 64.0% (3588/7800). Hatching rate in Group A was 36.0±5.29 % (82/230) compared to 40.0% (1435/3588) in the control. There were significant differences ($p< 0.05$) between Group A and its control in both fertilization rate and hatching rate.

Group B showed 25.8±4.1% triploidy. Mean fertilization rate in replicates of this group was 22.0±4.3% (1716/7800), whereas that in control was 35.0% (2730/7800). Mean hatching rate in replicates for group B was 25.0±3.0% (422/1716), while that in its control was 39.0% (1064/2730). There were significant differences ($p< 0.05$) between group B and its control in the both fertilization rate and hatching rate.

All embryos produced in group C (except in the control) died at the onset of cell division. Group D showed 10.8±2.4% triploidy. Replicates run for this group showed 21.0±3.6% (1638/7800) fertilization rate and 35.0±4.0% (578/1638) hatching rate. Control for Group D showed 42.0% (3276/7800) fertilization rate and 40.0% (1310/3276) hatching rate. There were significant differences ($p< 0.05$) between group D and its control in both fertilization rate and hatching rate.

No signs of triploidy were seen in groups E and F. Fertilization rate in replicates run for these groups were 15.3 ± 2.5% (1196/7800) and <1.0% (75/7800), respectively while those in their controls were 63.0% (4914/7800) and 78.0% (6084/7800), respectively. Hatching rate in the experimental groups E and F were 23.3±3.5% (276/1196) and 25.0% (18/75) respectively whereas in control group E it was 30.0% (1474/4914) and in control group F it was 30.0% (1825/6084). There were significant differences ($p< 0.05$) between both group E and group F and theirs control in both fertilization rate and hatching rate. Embryos produced from other replicates in group F died at the onset of cell division. Summary of results is shown in Table 1. Fingerlings produced in groups D and E showed 20.0-30.0% morphological deformities (curved tail, swollen stomach etc.).

Statistical comparison of triploidy rate in all groups showed that there were significant differences ($p< 0.05$) between groups A, B and D. Highest rate of triploidy was observed in group A and then groups B and D, respectively.

Table 1. Characteristics of thermal shocks, fertilization, hatching and triploidy rate in grass carp

Group	Shock temp. (ºC)	Starting time after fertilization (min)	Duration of shock (min)	Fertilization (%) (No. of embryos)	Hatching (%) (No. of larvae)	No. of larvae and fishes examined	No. of triploid larvae and fishes	Triploid yeild (%)
A	7	2.0	30	<3.0 (230/7800)	38.0 (87/230)	20	13	65.0
	"	"	"	" (230/7800)	40.0 (92/230)	20	12	60.0
	"	"	"	" (230/7800)	30.0 (69/230)	19	11	57.9
	Control	-	-	46.0 (3588/7800)	40.0 (1435/3588)	10	-	-
B	7	2.5	30	27.0 (2106/7800)	22.0 (463/2106)	23	7	30.4
	"	"	"	20.0 (1560/7800)	25.0 (390/1560)	18	4	22.2
	"	"	"	19.0 (1482/7800)	28.0 (415/1482)	32	8	25.0
	control	-	-	35.0 (2730/7800)	39.0 (1064/2730)	10	-	-
C	7	4.0	30	0.0	-	-	-	-
	"	"	"	0.0	-	-	-	-
	"	"	"	0.0	-	-	-	-
	control	-	-	70.0 (5460/7800)	22.0 (1201/5460)	10	-	-
D	38	4.0	1.0	18.0 (1404/7800)	35.0 (491/1404)	32	4	12.5
	"	"	"	20.0 (1560/7800)	31.0 (483/1560)	50	6	12.0
	"	"	"	25.0 (1950/7800)	39.0 (760/1950)	25	2	8.0
	control	-	-	42.0 (3276/7800)	40.0 (1310/3276)	10	-	-
E	40	4.0	1.0	13.0 (1014/7800)	27.0 (273/1014)	25	0	-
	"	"	"	15.0 (1170/7800)	20.0 (234/1170)	32	0	-
	"	"	"	18.0 (1404/7800)	23.0 (323/1404)	24	0	-
	control	-	-	63.0 (4914/7800)	30.0 (1474/4914)	10	-	-
F	42	4.0	1.0	<1.0 (75/7800)	25.0 (18/75)	10	0	-
	"	"	"	0.0	-	-	-	-
	"	"	"	0.0	-	-	-	-
	control	-	-	78.0 (6084/7800)	30.0 (1825/6084)	10	-	-

Comparison of chromosome numbers in triploid and diploid individuals showed that diploids had 2N= 48 chromosomes (Fig. 1), and triploids had 3N= 72 chromosomes (Fig. 2).

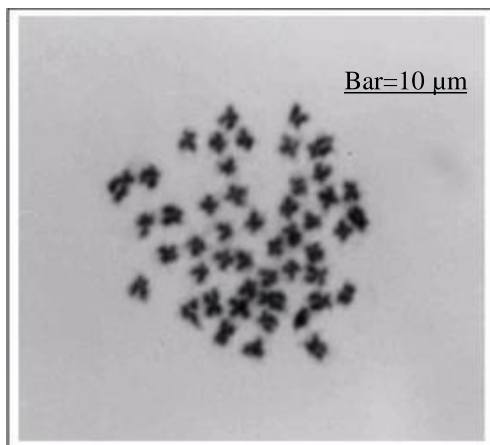

Fig 1. Chromosome spread prepared from diploid grass carp (2n = 48)

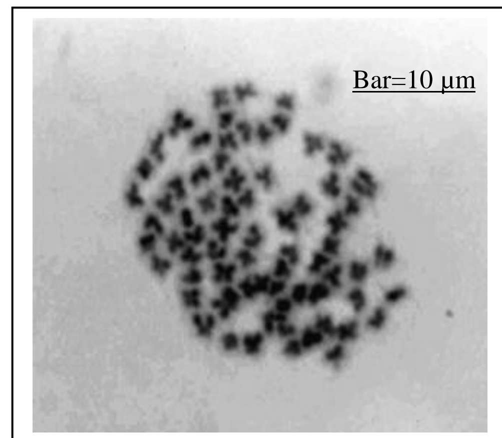

Fig 2. Chromosome spread prepared from triploid grass carp (3n = 72)

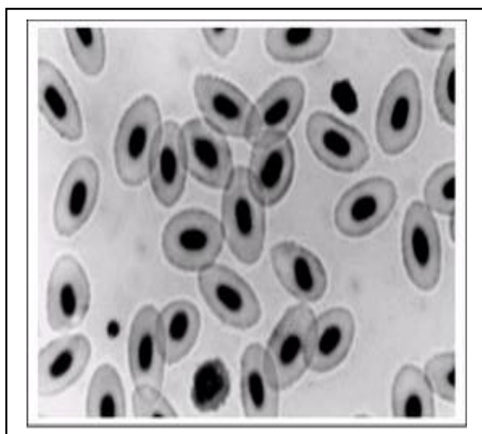

Fig 3. Blood smear prepared from diploid grass carp

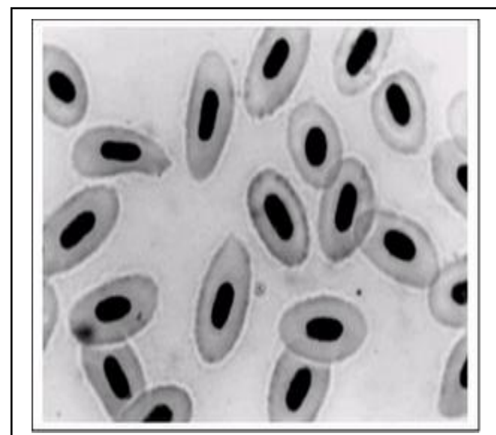

Fig 4. Blood smear prepared from triploid grass carp

Comparison of area and volume in erythrocytes (Fig. 5) and their nuclei (Fig. 6) between diploid (Fig. 3) and triploid (Fig. 4) grass carp are as follows:
Mean and standard deviation of large diameter (µm) of erythrocytes in diploid and triploid individuals were 10.7 ± 0.66 and 14.68 ± 0.68, respectively and large diameter (µm) of nuclei in these individuals were 4.6±0.31 and 6.14±0.4, respectively. There were significant differences ($p < 0.05$) between erythrocytes large diameter in diploid and triploid individuals. Significant differences ($p < 0.05$) were also detected in the large diameter of their nuclei. Mean and standard deviation of small diameter of erythrocytes in diploid and triploid individuals were 6.74±0.38 and 8.68±0.58, respectively and small diameter of nuclei in these individuals were 2.9±0.22 and 3.40±0.28 µm, respectively. There were significant differences ($p < 0.05$) between small diameter of erythrocytes in diploid and triploid individuals, but no significant differences in small diameter of their nuclei ($p > 0.05$). Mean and standard deviation of surface area of erythrocytes in diploid and triploid individuals were 56.45±4.45 and 101.52±8.80 µm², respectively and surface area of nuclei in these individuals were 10.5±0.90 and 16.33±1.33 µm², respectively. There were significant differences ($p < 0.05$) between erythrocytes surface area in diploid and triploid individuals, as in their nuclei area. Mean and standard deviation of volume of erythrocytes in diploid and triploid individuals were 255.00 ± 31.00 and 600.45±88.00 µm³, respectively and volume of nuclei in these individuals were 20.5±2.94 and 37.08±5.56 µm³, respectively. There were significant differences ($p < 0.05$) between erythrocytes volume in diploid and triploid individuals, as in their nuclei volume.

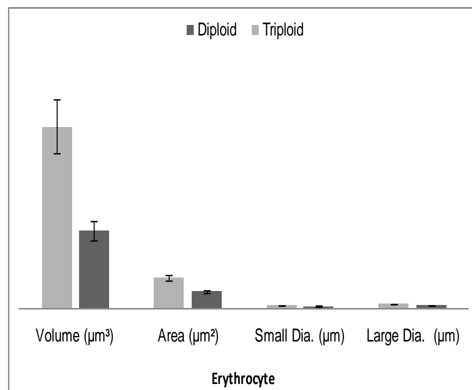

Fig.5. Comparison of mean values of large and small diameters, area and volume of erythrocytes of diploid and triploid grass carp

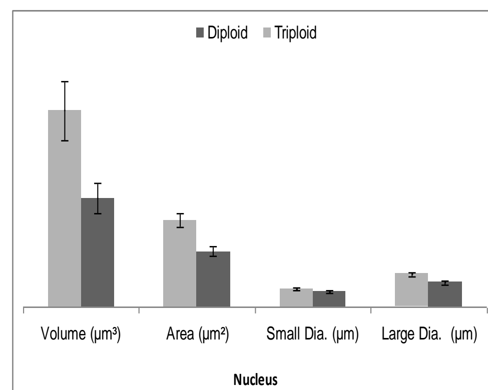

Fig.6. Comparison of mean values of large and small diameters, area and volume of nuclei of diploid and triploid grass carp

Ratios of surface area (1.80) and volume (2.35) of erythrocytes show that values for triploids are more than that for diploids. These ratios in nuclei of triploids were 1.55 and 1.80, respectively (Table 2).

Table 2. Comparison of mean values of measurements conducted on erythrocytes and their nuclei in diploid (2n) and triploid (3n) grass carp

Erythrocyte	Diploid	Triploid	Ratio of triploid to diploid
Large diameter (µ)	10.70 ± 0.66[a]	14.68 ± 0.68[a]	1.37
Small diameter (µ)	6.74 ± 0.38[a]	8.68 ± 0.58[b]	1.30
Large D. / Small D.	1.58	1.67	1.05
Area (µ²)	56.45 ± 4.45[a]	101.52 ± 8.80[b]	1.80
Volume (µ³)	255.00 ± 31.00[a]	600.45 ± 88.00[b]	2.35
Nucleus	**Diploid**	**Triploid**	**Ratio of triploid to diploid**
Large diameter (µ)	4.6 ± 0.31[a]	6.14 ± 0.4[b]	1.33
Small diameter (µ)	2.9 ± 0.22[a]	3.4 ± 0.28[b]	1.17
Large D. / Small D.	1.58	1.8	0.78
Area (µ²)	10.5 ± 0.9[a]	16.33 ± 1.33[b]	1.55
Volume (µ³)	20.5 + 2.94[a]	37.08 ± 5.56[b]	1.80

DISCUSSION

The feeding habits of grass carp were well known and it was thought to have great potential as a biological weed control agent (Opuszynski & Shireman, 1995). However, there was concern that the grass carp could reproduce in the wild and become an environmental nuisance, destroying valuable areas such as wetlands, swamps and waterfall feeding grounds. Because of these environmental concerns, early research focused on developing sterile populations. Attempts included producing single-gender populations (Boney et al. 1984), creating sterile hybrids (Marian & Krasznai, 1978) and removing gonads (Underwood et al. 1986). Success was limited because these methods were seldom 100 percent effective and verification of sterility was difficult. Then researchers and commercial producers began treating eggs with heat, cold or pressure shock to induce triploidy in fertilized eggs (Allen & Wattendorf, 1987). The extra chromosomes result in sterility. Triploid and diploid grass carp appear to consume similar quantities of aquatic plants and to have similar feeding habits, preferring succulent young plants.

Triploidy is induced by suppression of the second meiotic division in the fertilized eggs (Thorggard, 1983). Several factors including type of shock, timing of shock treatment after fertilization, and duration of shock can affect survival and triploidy yield. Long duration of shock treatment significantly decreases survival (Cassani & Caton, 1985). Also short durations of shock treatments may not be effective in inducing triploidy.

Highest percentage of triploidy (98.0%) in grass carp induced by hydrostatic pressure of fertilized eggs was reported by Cassani and Caton (1986), however using heat shock treatment in their study showed 66.7% triploidy. Thompson et al. (1987) produced 87.0% triploidy in grass carp using heat shocks. Increased percentage of triploidy produced by Thompson et al. (1987) may be due to the longer duration of heat shock (3-5 min) and quicker starting time (1 min) after egg - sperm activation for the given shock.

Lower fertilization rate observed in group A can be related to negative effect of cold shock on eggs and dilatory stripping time from female breeder. Also, fertilization rate was low in control of this group (46.0%). Despite the lower survival and less number of produced fingerlings, the percentage of induced triploidy was high (maximum percentage), thus indicating suitable timing of shock treatment after egg - sperm activation. Suitable timing of shock treatment after fertilization is effective in retention of the second polar body therefore shock treatment must coincide near or at the time of the second meiotic division (Babrova, 1969). The best results for induction of gynogenesis in grass carp were achieved by means of thermal shocks 2 - 5 min after fertilization (Stanley, 1979). According to Purdom (1969) apart from starting time of shock treatment after fertilization, temperature and duration of shock also must be accurate.

Fertilization rate in group B and its control were low. In this group dilatory fertilization (10 min after stripping) may have affected low fertilization rate. As Cassani and Caton (1986) found that delaying fertilization for 5-20 min after ovulation resulted in decreasing the activity of produced eggs. Delayed fertilization can adversely affect hatching and survival rate. Apart from poor survival and less number of produced fingerlings, about 25.8± 4.1% triploidy was induced in this group.

No larvae were hatched in group C (except for control) that may be due to further stripping from a fish. The first stripping was selected for control and the second stripping (about 70 min after the first stripping) was selected for the treatment and its replicates. The mortality of larvae from this treatment and its replicates may be associated to quality of eggs used from a brood fish that was stripped for a second time, but control of this group showed high fertilization (70.0%). These results for group C are similar to results of research conducted by Cassani and Caton (1985) in which the female broodfish were stripped a second time 51 min after the first stripping.

Fertilization rate was low in heat shock treatments as well as in control run for group D and this may be associated with quality of used eggs. Cassani and Caton (1985) and Varadaraj and Pandian (1990)

found that premature or overripe eggs can negatively affect fertilization rate. Stripping time is another important factor in increasing fertilization, survival and triploidy induction (Cassani & Caton, 1985). Zonneveld (1984) reported that if stripping is delayed in grass carp, eggs become overripe, resulting in poor fertilization and hatching. As insemination and retention of second polar body takes place in eggs, quality and health of breeders especially females and their eggs is important for genetic manipulations. Kjorsvik *et al.* (1990) announced that propagation of female fish is very complex and difficult, and it could include meiosis division, RNA production and its expression, vitellogenesis, ovulation, fertilization, response to hormone, size and egg quality.

Triploidy rate was 10.8 ± 2.4% in group D. Some of the larvae produced in this treatment showed morphological deformities and this may be due to the negative impacts of heat shocks (Cassani & Caton, 1986). Thompson *et al.* (1987) reported the presence of abnormal fish in treatments resulted in low percentage of triploidy.

Fertilization rate was low in group E and its experimental replicates and relatively high in the control. Negative effects of heat shocks may be one of the factors responsible for low fertilization (Cherfas *et al*. 1994).

In one of the replicates used for group F less than 1% fertilization was achieved, however mortality occurred in all embryos produced in all the other replicates of this group. Negative impacts of heat shocks may be responsible for this. Fertilization rate was 78% in the control treatment of group F.

In general, high temperature was responsible for mortality in eggs that received heat shock treatment. However heat shocks at lower temperature showed increased survival and decreased triploidy (Thorggard & Jazmin, 1981; Recoubratsky et al. 1992). No triploidy larvae were produced in groups E and F. It seems that heat shocks with high temperature negatively affected fertilization and hatching rates.

For verification of triploid individuals, karyotyping method (Beck *et al*. 1980) and measurement of erythrocytes and their nuclei dimensions were used. The chromosome number of diploid and triploid individuals were 2N=48 and 3N=72, respectively.

Nuclear size analyses are based on the fact that triploid nuclei, with the extra set of chromosomes, are approximately 50% larger than nuclei of similar diploid cells (Swarup, 1959). Erythrocytes or red blood cells are easily sampled in fish and are relatively consistent in size which make them appropriate for this type of analysis as well (Wolters *et al*. 1982; Beck & Biggers, 1983). On the other hand, microscopic measurement of surface area and volume of stained red blood cells nuclei was the method of choice for simple and relatively inexpensive analyses of triploid grass carp (Wattendorf, 1986). In the present experiment, 30 days old grass carp were used, because blood in smaller grass carp (14 days old or 20 mm total length) had a high percentage of hemoblasts and immature erythrocytes that had nuclei larger than mature erythrocyte nuclei for both diploids and triploids (Wattendorf, 1986).

In the present study, cold shocks were more effective in inducing triploidy in grass carp as compared to heat shocks. Increased tripolidy with cold shocks may be due to the susceptibility of eggs to cold shocks and duration of shock treatment may be the factor responsible for increased triploidy with cold shocks (Cassani & Caton, 1985). Generally, the number of larvae produced with heat shocks was more than those produced with cold shocks. Lower temperature used in heat shocks resulted in higher deformity and lower percentage of triploidy.

ACKNOWLEDGEMENTS

This study was supported by the Iranian Fisheries Research Organization. Our thanks are due to the deputy (Mr. Reza Khomeirani) of Shahid Ansari Hatchery and Fish breeding department. Also, we are grateful from Seyyed Kazem Jalilzadeh Moghimi, Alireza Kashani Sabet, Mohammad Kazmi, Afshin Adibpasand and Ms. Bahareh Haghighi for their sincere collaboration.

REFERENCES

Allen, S.K.Jr., Wattendorf, R.J (1987) Triploid grass carp Ctenopharyngodon idella: Status and management implications. *Fisheries*. 12 (4), 20-24.

Babrova, Yu.P (1969) On development of the gonads and the process of fertilization in the white amur Ctenopharyngodon idella. Pages 123-128 in Cherfas, B.L., editor. Proceedings of the Genetics selection and hybridization of fish. Akademii Nauk. SSSR (in Russian).

Beck, M.L., Biggers, C.J., Dupree, H.K (1980) Karyological analysis of *Ctenopharyngodon idella*, *Aristichthys nobilis*, and their F_1 hybrid. *Transaction of the American Fisheries Society.* 109, 433-438.

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

Boney, S.E., Shelton, W.L., Yang, S.L., Wilken, L.O (1984) Sex reversal and breeding of grass carp. *Transaction of the American Fisheries Society.* 113, 348-353.

Buck, H (1979) Optimism swells with the possibility of a sterile hybrid grass carp. *Fisheries*, 4(5), 31.

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

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

Cherfas, N.B., Gomelsky, B., Ben-Dom, N., Peretz, Y., Hulata, G (1994) Assessment of triploid common carp *Cyprinus carpio* L. for culture. *Aquaculture.* 127, 11-18.

Clippinger, D., Osborne, J.A (1984) Surgical sterilization of grass carp, a nice idea. *Aquatics*. 6, 9-10.

Kjorsvik, E., Mangorjensen, A., Holmefjord, I (1990) Egg quality in fishes. *Advances in Marine Biology.* 26, 71-113.

Kligerman, A.D., Bloom, S.E (1977) Rapid chromosome preparation from solid tissues of fishes. *Journal of the Fisheries Research Board of Canada.* 34, 266-269.

Krasznai, Z., Marian, T., Jeney, Z., Jeney, G., Zsigri, A (1984) Effect of triploidy on the blood cell size of hybrid grass carp *Ctenopharyngodon idella* Val. *Aquacultura Hungarica.* IV, 17-24.

Lemoine, L.H., Smith, T.L (1980) Polyploidy induced in brook trout by cold shock. *Transaction of the American Fisheries Society.*109, 626-631.

Marian, T., Krasznai, Z (1978) Kariological investigation of *Ctenopharyngodon idella* and *Hypophthalmichthys nobilis* and their cross-breeding. *Aquacultura Hungarica.* 1, 44-50.

Mitchell, A.J., Kelly, A.M (2006) The public sector role in the establishment of grass carp in the United States. *Fisheries*. 31(3), 113-121.

Mitzner, L (1978) Evaluation of biological control of nuisance aquatic vegetation by grass carp *Ctenopharyngodon idella* Val. *Transaction of the American Fisheries Society.* 107, 135-191.

Opuszynski, K., Shireman, J.V. (1995) *Herbivorous fishes, culture and use for weed management*. CRC Press. 223 pp.

Purdom, C.E (1969) Radiation-induced gynogenesis and androgenesis in fish. *Heredity.* 24, 431-444.

Purdom, C.E (1972) Induced polyploidy in plaice and its hybrid with the flounder. *Heredity*. 29, 11-23.

Recoubratsky, A.V., Gomelsky, B.I., Emelianova, O.V., Pankratyeva, E.V (1992) Triploid common carp *Cyprinus carpio* L. produced by heat shock with industrial fish farming technology. *Aquaculture.* 108, 13-19.

Rothbard, S (1981) Induced reproduction in cultivated cyprinids-the common carp and the group of Chinese carps. I. The technique of induction, spawning and hatching. *Bamidgeh*. 33, 103-121.

Rottmann, R.W., Shireman, J.V., Chapman, F.A. (1991) Hormone preparation, dosage *calculation, and injection techniques for induced spawning of fish*. SRAC Publication. No. 425.

Sezaki, K., Kobayasi, H (1978) Comparison of erythrocytic size between diploid and tetraploid in spinous loach Cobitis biwae. *Bulletin of the Japanese Society of Scientific Fisheries*. 44, 851-854.

Stanley, J.G (1976) Production of hybrid, androgenetic, and gynogenetic grass carp. *Transaction of the American Fisheries Society*. 105, 10-16.

Stanley, J.G., Miley, W.W., Sutton, D.L (1978) Reproductive requirements of the likelihood for naturalization of escaped grass carp *Ctenopharyngodon idella* in the United States. *Transaction of the American Fisheries Society.* 107, 119-128.

Stanley, J.G (1979) Control of sex in fishes, with special reference to the grass carp. pages 201-242 in Shireman, J. V., editor. Proceedings of the Grass Carp Conference. University of Florida, Institute of Food and Agricultural Sciences, Gainesville, FL.

Sutton, D.L (1977) Grass carp *Ctenopharyngodon idella* in North America. *Aquatic Botany.* 3, 157-164.

Swarup, H (1959) The effect of triploidy on the body size, general organization and cellular structure in *Gasteroteus aculeatus* (L.). *Journal of Genetics.* 56, 143-155.

Thompson, B.Z., Wattendorf, R.J., Hestand, R.S., Underwood, J.L (1987) Triploid grass carp *Ctenopharyngodon idella* production. *Progressive Fish-Culturist.* 42, 213-217.

Thorggard, G.H (1983) Chromosome set manipulation and sex control in fish. Pages 405-434 in W.H. Hoar, D.J. Randall, E.M. Donaldson, editors. Fish Phisiology. Academic press, Newyork, Newyork, USA.

Thorggard, G.H., Jazmin, M.E (1981) Polyploidy induced by heat shock in rainbow trout *Oncorhynchus mykiss*. *Transaction of the American Fisheries Society.* 110, 546-550.

Underwood, J.L., Hestand, R.S., Thompson, B.Z (1986) Gonad regeneration in grass carp following bilateral gonadectomy. *Progressive Fish-Culturist.* 48, 54-56.

Varadaraj, K., Panadian, T.J (1990) Production of all female sterile triploid *Oreochromis mossambicus*. *Aquaculture.* 84, 117-123.

Wattendorf, R.J (1986) Rapid analysis of ploidy via a Coulter Counter with channelyzer, with emphasis on grass carp *Ctenopharyngodon idella* Val. *Progressive Fish-Culturist.* 48, 125-132.

Wolters, W.R., Chrisman, C.L., Libey, G.S (1982) Erythrocyte nuclear measurements of diploid and triploid channel catfish, *Ictalurus punctatus* (Rafanesrue). *Journal of Fish Biology.* 20, 253-258.

Zimpfer, S.P., Bryan, C.F., Pennington, C.H (1987) Factors associated with the dynamics of grass carp *Ctenopharyngodon idella* larvae in the lower Mississippi River valley. *American Fisheries Society Symposium*. 2, 102-108.

Zonneveld, N (1984) The spawning season and the relation between temperature and stripping time of grass carp Ctenopharyngodon idella Val. (In Egypt). *Bamidgeh.* 36, 21-28.

القای تریپلوئیدی در ماهی کپور علفخوار
Ctenopharyngodon idella Valenciennes, 1844:
مقایسه شوکهای سرمایی و گرمایی

م. حسن زاده صابر*، م. پورکاظمی

(تاریخ دریافت: ۹۰/۶/۱۱ - تاریخ پذیرش: ۹۰/۱۲/۲۶)

چکیده

تریپلوئیدی در کپور علفخوار با هدف مقایسه شوکهای سرمایی و گرمایی بر روی تخمهای لقاح یافته این ماهی القا شد. سپس دو روش ساده برای تایید تریپلوئیدی در کپور علفخوار توضیح داده شد. شوک سرمایی (۷ درجه سانتیگراد) در سه تیمار بمدت ۳۰ دقیقه در زمانهای ۲، ۲/۵، و ۴ دقیقه بعد از لقاح داده شد. شوک سرمایی در زمان ۲ دقیقه بعد از لقاح، بالاترین میزان تریپلوئیدی (۶۰/۹٪) را نشان داد. شوکهای گرمایی با درجه حرارت ۳۸، ۴۰ و ۴۲ درجه سانتیگراد در ۴ دقیقه بعد از لقاح بمدت ۱ دقیقه داده شد. ۱۰/۸٪ لاروهای تولید شده در شوک گرمایی ۳۸ درجه سانتیگراد تریپلوئید بودند ولی هیچ لارو تریپلوئیدی در دیگر تیمارهای شوک گرمایی ظاهر نشد. تایید تریپلوئیدی در کپور علفخوار با استفاده از بررسی تعداد کروموزومها و اندازه گیری مساحت و حجم اریتروسیت در بچه ماهیان انگشت قد انجام پذیرفت. نسبت ابعاد اریتروسیت ها و هسته آنها در تریپلوئید ها به دیپلوئید ها بترتیب ۲/۳۵ و ۱/۸ بود. ماهیان تریپلوئید باالای ۵۰٪ نگهداری گردیدند. هر ماهی قبل از نگهداری کنترل و تایید شد تا ۱۰۰٪ ماهی تریپلوئید ذخیره شود. مقایسه نتایج بدست آمده از بکارگیری شوکهای سرمایی و گرمایی اشاره دارد که شوکهای سرمایی به نسبت شوکهای گرمایی تاثیر بیشتری در القای تریپلوئیدی در ماهی کپور علفخوار دارد.

*مولف مسئول