INDUCTION OF POLYPLOIDY IN ISRAELI CARP

FRANK MERIWETHER, II, Department of Fisheries and Allied Aquacultures, Auburn University, AL 36849

Abstract: Israeli carp *(Cyprinus carpio)* eggs were cold-shocked after fertilization at various temperature regimes and durations. Nine months later their blood was sampled for analysis. Stained erythrocyte nuclei were measured with an ocular micrometer to calculate mean relative areas. Polyploidy appeared to have been induced in each cold-shock group, but no group appeared to consist entirely of polyploids. Gonadal examination showed no apparent differences between the control group and the cold-shocked groups.

Proc. Ann. Conf. S.E. Assoc. Fish & Wildl. Agencies 34:275-279

The management of certain prolific fish species is possible when stocking populations of sterile fish. Sterility may decrease mortality and increase meat quality in certain fish species upon sexual maturity (Refstie et al. 1977). Sterile fish should also grow at faster rates than normal fish, as metabolic energy ordinarily used for gonad development could be used for somatic growth (Allen and Stanley 1978).

Induced polyploidy may be one method to produce permanently sterile fish. Fish with a triploid (3N) complement of chromosomes are expected to be sterile because homologous chromosomes would be unable to synapse in gametogenesis.

Temperature shocking of fish eggs to induce ploidy changes was reviewed by Allen and Stanley (1978); very little or no published information is available on temperature shocking of fertilized Israeli carp eggs. A sudden cold shock was more effective than a heat shock in inducing triploidy in the stickleback (Swarup 1958), as high mortality occurred with heat shock treatments. Also, the cold-shock mortality rate in stickleback embryos was decreased by reducing the duration of treatment (Swarup 1958). All-triploid offspring may be produced by crossing a normal diploid individual with a tetraploid (Refstie et al. 1977). The induction of either a triploid or a tetraploid (4N) state in fish can possibly be selected for, depending primarily on the time (post-fertilization) at which the cold shock treatment is begun (De Jong 1957).

Nuclear sizes are related to the number of chromosome sets present in a cell, especially as concerns polyploidy within a single species (Bachmann and Cowden 1967). The volumes of triploid and diploid nuclei should theoretically be the ratio 3:2 (Purdom 1972) due to increased chromatin content (De Jong 1957). Erythrocyte nuclear area has been found to be significantly correlated with the number of nucleoli (Cherfas 1966) and DNA content (Bachmann and Cowden 1967) in erythrocyte nuclei of various fish species. Areas calculated from dimensions of stained erythrocyte nuclei may be compared on a proportional basis with those of other treatments (Cimino 1963, Cherfas 1966, Bachmann and Cowden 1967). Purdom (1972) stated that the ratio of erythrocyte nuclear areas of triploid:diploid fish should be 1.3:1. However, research with hybrid Chinese carp (Stanley 1976) and goldfish (Cherfas 1966) indicates that the ratio should be 1.4-1.5:1.

METHODS

In May 1979, Israeli carp brooders were selected for hypophysation treatments. Human chorionic gonadotropin and dried common carp pituitaries were given at the rates shown in Table 1. All brooders weighed between 1.3 and 2.3 kg.

Eggs were collected by hand-stripping. Milt from a single male fertilized the eggs stripped from individual females. After ambient temperature water was added, bentonite

Table 1. Injection dosages given to Israeli carp brooders, per kg of brooder.

Sex	Stimulating Dose	Resolving Dose[1]/
Female	45 I.U. HCG[2]/ + 0.45 mg carp pituitary	136 I.U. HCG + 0.91 mg carp pituitary
Male	None given	23 I.U. HCG + 0.91 mg carp pituitary

[1]/Resolving dose given 9 hours after female stimulating dose.

[2]/HCG = Human chorionic gonadotropin.

was added to reduce clumping of eggs.

The various shocking regimes (treatments) that the fertilized eggs were subjected to are shown in Table 2. Eggs were randomly apportioned and quickly submersed in the cold water (De Jong 1957). One group of eggs was dipped in 0.1 percent colchicine prior to the cold shock. Cold water temperatures were produced by Frigid Units® suspended in fiberglass troughs. Eggs were suspended in the troughs in plastic buckets, each having 3 screened side openings to provide for adequate water flow through the eggs.

Following the cold shock, eggs were immediately transferred to MacDonald jars for incubation. After hatching, fry were collected in 38-l aquaria. Hard boiled egg yolk was fed to fry before stocking outdoors into 4,200 l plastic pools. Each pool had been treated with 1 ppm Dylox, and had a sack containing sheep manure suspended in the water to stimulate plankton development. Trout diet pellets were fed to the fingerlings. Only those treatments with "good" survival through the hatching stage (Table 2) were retained for further analysis; treatments D, E, and F were not analyzed because of excessive cold-shock mortality.

Twenty-five fish were randomly sampled from each of the 4 remaining treatments for collection of blood smears. All fish were 11 to 14 cm in length. The caudal peduncle was severed and blood collected in a nonheparinized capillary tube to prevent possible effects of heparin on changes in nuclear size (John Grizzle, personal communication)[1]. Blood smears were fixed in methanol for at least one minute before being stained. The length and width of 15 nuclei from each fish were measured with an ocular micrometer having a precision of $\pm$ 0.25 µm at 1000X total magnification. Mean areas of erythrocyte nuclei for the 4 treatments were compared using Duncan's multiple range test. Fifteen fish from each treatment were also randomly selected and their gonads examined using the aceto-carmine squash technique (Guerrero and Shelton 1974).

RESULTS

A higher proportion of physical abnormalities was observed in the cold-shocked carp than in the control group. Some cold-shocked sac fry had lordosis, scoliosis, twisted caudal peduncles or petechial yolk sacs. Many fingerlings had short opercles and gnarled, missing or eroded fins.

Analytical results of the 4 shocking treatments that had "good" survival are shown in Table 3. Each of the 4 treatment groups had a mean erythrocyte nuclear area significantly

[1]Assistant Professor, Department of Fisheries and Allied Aquacultures, Auburn University, AL 36849.

Table 2. Cold-shocking regimes used on fertilized Israeli carp eggs to induce polyploidy.

Group	Female brooder no.	Time of cold shock (min. post-fertilization)	Duration of cold shock (min.)	Ambient temperature (°C)	Cold shock temperature (°C)	Survival through hatching stage
A	1	15	30	18	6	good
B[1]/	1	--	--	18	-	good
C	2	15	20	19	2	good
D	2	15	40	19	2	poor
E	2	15	60	19	7	poor
F	2	15	90	19	7	poor
G[2]/	3	60	60	20	6	good

[1]/Control group.

[2]/Eggs were dipped in 0.1 percent colchicine at 15 minutes post-fertilization for 40 minutes, then cold shocked.

Table 3. Erythrocyte nuclear areas[1]/ of cold-shocked Israeli carp as measured by an ocular micrometer.

Group	No. of nuclei[2]/	Mean area[1]/ (μm^2)	Standard deviation	Mean area ratio [3]/	Duncan's range test[4]/
A	375	4.632	0.637	1.29	a
B (control)	375	3.579	0.401	1.00	b
C	375	4.204	0.541	1.17	c
G	375	3.693	0.514	1.03	d

[1]/Area = length x width (μm^2).

[2]/Fifteen nuclei from each of 25 fish per treatment.

[3]/Mean area ratio = mean area of treatment divided by mean area of control group.

[4]/Treatments with different letters are significantly different from each other ($P < .05$).

different ($P < .05$) from each other. The control group (group B) had the smallest mean nuclear area, which was expected if polyploidy was induced in the cold-shocked groups. The largest ratio (1.29:1) of the mean nuclear area of any group to the control group was that of group A to (control) group B (Table 3).

Duncan's multiple range test (Table 3) and an analysis of variance demonstrated that the variance within treatments was less than the variance between treatments ($F = 315.7$, 3 d.f.). The 4 treatment variances were not homogeneous as shown by Bartlett's test, so the data were transformed to logarithms for a second analysis (Zar 1974). Duncan's multiple range test again showed significant differences between any 2 treatment means ($P < .05$).

Carp fry originating from eggs dipped in colchicine (group G, Table 3) had a mean nuclear area only slightly larger than the control group ($P < .05$). Size variation was similar to that of other groups (Table 3).

The estimated coefficient of determination (r^2) value of 0.388 indicated that a significant portion of the total variation in area values could be explained by treatment effects. However, most of the area variation (61.2%) was due to mitotic or random fluctuations, or differences caused by uncontrollable factors.

Gonads from each cold shock treatment possessed either primary oocytes or testicular tissue. Also, gonadal shape and appearance in cold shock groups were not obviously different from the control group.

DISCUSSION

Induction of polyploidy was accomplished in Israeli carp at several different temperature regimes. Effective temperature shocks appeared to be those close to lethal temperature conditions for the eggs, which were 2° C for 40 minutes and 7° C for 60 minutes, when cold shocked 15 minutes post-fertilization. Group A had the largest mean nuclear area, indicating that polyploidy had occurred at a higher rate in this group than in other cold shock groups. Group A eggs were cold shocked for 30 minutes at 6° C, starting 15 minutes post-fertilization. However, the relatively low ratio of the mean nuclear area of group A to that of the control (1.29:1) indicated that a complete state of triploidy may not have been induced in any group of Israeli carp. Therefore, those carp in treatments that had high mean nuclear areas may possess mosaic cell constitutions, i.e. contain more than 1 level of ploidy (Allen and Stanley 1978). As production of an all-triploid population of carp was not accomplished, further research is needed to define effective temperature shock conditions for induction of polyploidy. Those mosaic individuals that appear to have been produced in this experiment may be eventually capable of reproduction. Sterility in such fish may be shown only when the cold-shocked carp grow to a mature size.

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