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**Optimization of Chromosome Set and Sex
Manipulations in Common Carp, *Cyprinus carpio* L.**

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"OPTIMIZATION OF CHROMOSOME-SET AND SEX
MANIPULATIONS IN THE COMMON CARP *Cyprinus carpio* L."

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ABSTRACT

The optimum temperature shock timings for the suppression of meiosis II and the 1st cleavage were determined and standardized, using the relative unit of embryological age (τ_0). The highest production of 2n meiotic gynogenetic (or 3n) and of 2n mitotic gynogenetic (or 4n) larvae can be expected when heat shock is applied, respectively, at 0.10-0.25 τ_0 and at 1.5 τ_0 , after insemination. The optimum timings for cold shock initiation (investigated for meiosis II only) corresponds to 0.05-0.10 τ_0 and to 0.30-0.40 τ_0 , respectively. The dynamics of survival and of 2n gynogens production have opposing tendency in cold and heat shocked progenies, suggesting that different intracellular mechanisms are responsible for the effects of high and low temperatures. Hydrostatic pressure shock for induction of 2nd polar body gynogens was most successful when applied at 7500-8000 PSI for 1 min between 0.25-0.30 τ_0 (preliminary results). Meiotic and mitotic gynogens and 3n of Israeli common carp were mass produced (in heat shock experiments) and reared to adult stages. No advantage of culturing triploid common carp over diploid common carp was observed. All tetraploid fish obtained died, and the question whether or not it is possible to obtain adult tetraploid common carp remains unsolved.

The phenomenon of spontaneous diploidization in koi was shown to have a hereditary nature. Some transformation in early meiotic stages may, apparently, be responsible for unreduced eggs production.

Density-dependent growth affected gonadal differentiation. In general, stages are reached and passed at a younger age but at a larger size among more rapidly growing fish and vice versa. The methyltestosterone immersion treatments were ineffective in altering the sex ratio of any treatment group. Higher effectiveness of oral androgen treatment in recirculation water systems (than in the tanks with running water) was confirmed: the frequency of inverted males was 47-96% and 20-44%, respectively, in gynogenetic treatment groups under the two conditions.

OBJECTIVES OF THE ORIGINAL RESEARCH PROPOSAL

The general objective of the research proposal was the further development of the methods of induced gynogenesis, polyploidy and hormonal sex inversion in common carp *Cyprinus carpio* L., the important object of aquaculture. Each component has previously been accomplished for the common carp but the various phases lack optimization which has been an impediment to programmatic development and pragmatic application of these methods in the fish culture industry.

The main topics focused on in this research were:

1. Optimization of gynogenesis and polyploidy treatments for common carp, and, primarily, further development of the "temperature shock" treatments which are used for inducing chromosome-set diploidization in various stages of early embryogenesis. These investigations included:
 - a) Determination of the optimum temperature shock timing during the 2nd meiotic division and the 1st cleavage; evaluation of effectiveness of hydrostatic pressure for inducing 2nd polar-body retention; examination of the possibility to standardize shock timing by using the relative measure of embryological age τ_0 .
 - b) Specifying the protocols for production of gynogenetic and polyploid common carp progenies on a large scale;
2. Investigation of the phenomenon of spontaneous diploidization in ornamental (koi) common carp, including:
 - a) Study of previously obtained spontaneous gynogenetic offspring;
 - b) Testing of hereditary control of this phenomenon and its cytogenetic features.
3. Optimization of methods of hormone-induced sex reversal in common carp females, including:
 - a) Evaluation of the effectiveness of hormone-induced sex reversal by immersion treatment, as an alternative to oral administration of androgen;
 - b) Further development of a protocol for oral androgen treatment;
4. Production and culture evaluation of diploid and triploid unisexual (all-female) common carp progenies.

The investigations were carried out according to the project's program, and most of the planned tasks have been fulfilled. The following deviations from the research plan should be noted:

Induced gynogenesis and polyploidy

Evaluation of pressure shock effectiveness (U.S.A.) was delayed until year 3 because of technical complications in developing the equipment. Only preliminary trials were accomplished during the study. Karyological investigations (Israel) were substituted for less laborious flow cytometry analyses.

Investigations of spontaneous diploidization phenomenon (Israel)

Only part of the planned crossings were carried out due to death of some breeders.

Investigations on hormone-induced sex inversion (U.S.A.) were carried on bisexual common carp progenies, due to failure of obtaining unisexual gynogenetic progenies on a large scale.

Investigations on culture assessment of sterile triploid fish (Israel) were carried out on bisexual (but not unisexual) triploid progenies, since the latter have not been obtained; obtaining of unisexual diploid progenies (Israel) was delayed until year 3 due to the unfavourable weather conditions in 1992, thus the data obtained are restricted to the observations on fingerlings only (final results will be obtained in 1994, i.e., beyond project's termination).

BODY OF REPORT

Introduction

The BARD project "Optimization of chromosome set and sex manipulations in the common carp, *Cyprinus carpio* L." was aimed at further development of the methods for induced diploid gynogenesis, induced polyploidy and hormonal sex reversal techniques in the common carp. Theoretical and practical importance of these techniques were discussed in many reviews (e.g., Kirpichnikov, 1981; Thorgaard, 1983; Shelton, 1986, 1990; Nagy, 1987). The most important applied problems which could be solved by these methods are: obtaining unisexual diploid and/or triploid (sterile) progenies for the management of unwanted reproduction in grow-out ponds; increasing growth potential of carp in Israel by using all-female progenies; fast creation of homozygous progenies (with inbreeding coefficient =1); obtaining isogenic progenies, i.e., lines and clones with fixed valuable traits. Some important theoretical problems in the field of fish cytogenetics and selection (e.g., gene mapping, estimation of inbreeding depression, analysis of chromosome constitution of sex) can also be investigated by these methods. Although there are a large number of investigations on induced of gynogenesis, polyploidy and sex reversal in the common carp, some important questions concerning these methods were not clear, and the data obtained appeared contradictory in some cases. The present project was aimed at closing some of these gaps.

According to the project program, the investigations were carried out in two principal directions. One was devoted to further elaboration of the methods of induced gynogenesis and polyploidy, and also to culture evaluation of progenies obtained by these methods. These investigations were carried out primarily in Israel. The second dealt with optimization of sex reversal techniques for the common carp, needed for propagation of all-female gynogenetic lines. These investigations were carried out primarily in the U.S.A.

DESCRIPTION OF COOPERATION

The research team met during the initial year of funding (at a Symposium on Carp Genetics, held in Szarvas, Hungary in September 1990) when the plans for the coming spawning season were discussed. The U.S. principle investigator spent the period January-June 1992 in Israel on a sabbatical leave from his home institution. Collaboration with the Israeli team was valueable. During this period, different questions related to the project experiments were discussed, and the techniques associated with ploidy manipulation were practiced. Time was shared between the Dor Research Station and the Fish Breeding Center at Kibbutz Gan Shmuel. Appreciation is sincerely expressed to the personnel of both facilities, and to the University of Oklahoma for making this valuable experience possible.

In an effort to establish a common fish stock, the Dor-70 carp line was spawned in Israel in 1991 and some progeny were sent to the U.S. counterpart later that year. These have been nursed during the interim but did not mature soon enough to be used during the funding period.

EVALUATION OF THE RESEARCH ACHIEVEMENTS

The investigations have been carried out according to the project program, and the research objectives of the project were attained. The most important achievements are as follows:

1. In the investigations on induced gynogenesis and polyploidy, precise data concerning the efficiency of temperature shock at different phases of the 2nd meiotic division (heat and cold shocks) and the 1st cleavage (heat shock) in common carp were obtained; based on these data the optimum timing for suppression of the 2nd meiotic division and the 1st cleavage in common carp was established. The possibility for standardizing temperature shock timing (independently of pre-shock water temperature) using the relative unit of embryological age τ_0 was confirmed. Protocols for obtaining diploid meiotic and mitotic gynogens, tri- and tetraploid progenies in a large scale were specified.
2. Specific investigations on the nature of spontaneous diploidization in koi were carried out for the first time; the data obtained indicated that this phenomenon

has, at least in some cases a genetic determination, and that this property is heritable. Transformation, occurring at early meiosis stages, may be responsible for production of unreduced eggs.

3. In the investigations on hormone-induced sex reversal in common carp, the importance of growth history to the developmental relationship of the functional gonad was demonstrated. These factors need to be considered for sex reversal therapy. It was shown that growth dynamics affects the age/size relationship of critical events in ontogeny of the reproductive system. Efforts to manipulate the phenotypic expression of these genetically controlled events, must recognize and incorporate growth factors into protocols which propose to alter the usual phenotypic pathway.

Additional data concerning higher effectiveness of recirculated water systems than those with running water for sex inversion were obtained. Earlier observations showing the possible involvement of some soluble metabolite(s) of high activity (transformed from the treatment hormone) have been confirmed.

The importance of the methods of chromosome-set and sex manipulations for aquaculture has been noted above. The project yielded new data for optimizing the induced gynogenesis and sex inversion techniques in the common carp. The results obtained were already implemented in the studies on the Israeli Dor-70 common carp line. Two type of Dor-70 gynogens have been obtained in 1992-1993. Some of these (all-female) fish were transformed into phenotypic males by the androgen treatment. As a result, stocks of meiotic gynogenetic females and males, and mitotic gynogenetic females and males of Israeli common carp have been created for the first time. These, in turn, have provided the opportunity for a number of interesting further studies. Thus, meiotic gynogenetic inverted males (XX) have been crossed with regular Dor-70 females. Unisexual all-female progenies Dor-70 were obtained from this crossings on a large scale and are currently under testing. Mitotic gynogenetic Dor-70 females are planned to be used (after reaching sexual maturity) for obtaining genetically homogeneous progenies, i.e., clones. Mitotic gynogenetic inverted males (of expected inbreeding coefficient = 1) can be also used for producing topcrosses with regular females to benefit from the expected heterosis effect.

The results obtained were presented at the following forums: The 4th International Symposium on Genetics in Aquaculture (April-May 1991, Wuhan, China); International Satellite Symposium "Frontiers of Biotechnology in Agriculture 1991" (August 1991, Sea of Galilee, Israel); Japanese-Israeli Symposium on Aquaculture - JISA (November 1992, Haifa, Israel); Annual Meeting of the World Aquaculture Society (July 1992, Florida, USA); International Symposium "The Carp" (September 1993, Budapest, Hungary). Some results will also be presented at the 5th International Symposium on Genetics in Aquaculture (June 1994, Halifax, Canada).

LIST OF PUBLICATIONS

1. N.B. Cherfas, G. Hulata & O. Kozinsky, 1993. Induced diploid gynogenesis and polyploidy in ornamental (koi) carp, *Cyprinus carpio* L. 2. Timing of heat shock during the first cleavage. *Aquaculture*, 111: 281-290.
2. N.B. Cherfas, Y. Peretz, N. Ben-Dom, B. Gomelsky & G. Hulata. Induced diploid gynogenesis and polyploidy in ornamental (koi) common carp *Cyprinus carpio* L. 3. Optimization of the heat shock timing during the 2nd meiotic division and the 1st cleavage. *Theor. Appl. Genet.*, in press
3. N.B. Cherfas, Y. Peretz, N. Ben-Dom, B. Gomelsky & G. Hulata. Induced diploid gynogenesis and polyploidy in ornamental (koi) common carp, *Cyprinus carpio* L. 4. Comparative study on the effects of cold and heat temperature shocks. *Theor. Appl. Genet.*, in press.
4. N.B. Cherfas, B. Gomelsky, Y. Peretz, N. Ben-dom & G. Hulata, 1993. Induced gynogenesis and polyploidy in the Israeli common carp line Dor-70. *The Israeli J. of Aquaculture-Bamidgeh*, 45(2): 59-72.
5. N.B. Cherfas, B. Gomelsky, N. Ben-Dom & G. Hulata. Evidence for the heritable nature of spontaneous diploidization in common carp eggs. Submitted to *Aquaculture and Fisheries Management*.
6. N.B. Cherfas, B. Gomelsky, N. Ben-Dom, Y. Peretz & G. Hulata. Assessment of triploid common carp for culture. Submitted to *Aquaculture*.
7. B. Gomelsky, N.B. Cherfas, Y. Peretz, N. Ben-Dom & G. Hulata. Hormonal sex inversion in the common carp (*Cyprinus carpio* L.). Submitted to *Aquaculture*.