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http://www.aquaculturehub.org

ISSN 0792 - 156X

PUBLISHER:
Israeli Journal of Aquaculture - BAMIGDEH -
Kibbutz Ein Hamifratz, Mobile Post 25210,
ISRAEL
Phone: + 972 52 3965809
http://siamb.org.il

A Review of Ploidy Manipulations in Aquaculture: the Israeli Experience

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Key words: androgenesis, aromatase inhibitor, chromosome set, dimensionless factor τ_0, embryonic age, gynogenesis, neomales, ploidy manipulation, polyploidy

Abstract

Ploidy manipulation was introduced to Israel at the beginning of the 1980s. To improve existing methods, Israeli fish centers conducted intensive research in this field for more than two decades. Chromosome-set technology was adapted for ten fish species and varieties, including two marine fish. Presently, though methodology achievements are remarkable, practical implementation is applicable only for the common carp. The aim was to integrate gynogenesis and androgenic sex-reversal to generate neomales with special genetic traits in this economically important species that is extensively cultured in Israel. Carp neomales can serve as parents for production of fast-growing all-female populations. This paper describes ploidy manipulation progress and activities carried out in four Israeli fish centers. Future objectives include (a) implementation of the existing know-how to produce tilapia YY-males as broodfish for producing male-monosex populations, (b) dissemination and promotion of export to foreign markets of the advantageous female triploid grass carp and black carp, (c) continuation of genetic work with marine fish to develop breeds that could be advantageous for mariculture, and (d) reconsideration of the ploidy-manipulated koi as a genetic tool to be utilized to facilitate gene-mapping in carps.

Introduction

Early investigations of ploidy manipulation in fish have been reported by Stanley (1976) for grass carp, *Ctenopharyngodon idella* V., by Nagy *et al*. (1978) for common carp, *Cyprinus carpio* L. and by Chourrout (1980) for rainbow trout, *Salmo gairdneri* R. Techniques of chromosome-set manipulations were introduced to Israel in the early 80's of the last century.

The ploidy manipulation technology was brought to attention of the Israeli aquaculturists when a group of three Hungarian scientists, headed by Dr. J. Bakos from Fish Culture Research Institute in Szarvas, visited Israel to meet Israeli aquaculture scientists and fish farmers at Dor Aquaculture Research Station, and to participate in an round-table discussion on recent advancements in Hungarian aquaculture. One of the major topics that stimulated deep interest of the Israeli participants was a lecture on ploidy manipulations held in Hungarian and simultaneously translated into Hebrew.

In Israel, first attempts to introduce the technology of induction diploid gynogenesis and polyploidy, was carried out with tilapias in the Gan Shmuel Fish Breeding Center

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(Chourrout and Itskovich, 1983). Some years later, chromosome-set manipulation was intensively researched at Dor Station (Cherfas et al., 1990) in order to adopt the technology to Japanese ornamental (koi) carp.

In the last two decades four fish culture centers were involved in investigation of chromosome-set manipulations, the Aquaculture Research Station – Dor (Ministry of Agriculture), the YAFIT (R&D) Laboratory in kibbutz Gan Shmuel, the National Center for Mariculture (IOLR - Eilat) and the Laboratory of Fish Immunology and Genetics – Bar Ilan University.

Israeli scientists involved in this technology have investigated various aspects of ploidy manipulations during 25 years of research. The following subjects were selected for research: methods of shock application (type, level, duration, etc.), UV-irradiation (intensity, duration, conditions, etc.), embryonic age required for external intervention (τ_0 factor), and the use of genetic markers (colors, scalation, fin shapes, chromosome structures, etc.). All these factors are essential to identify and to achieve success at the process of ploidy manipulation. The possibility of storage carp eggs prior to fertilization for short durations was also investigated (Rothbard et al., 1996).

Although the methodologies and technology of ploidy manipulation have been well developed and well established for various fish species, the commercial implementation of the methods have been rather unsatisfactory due to several constrains. For instance, in spite of generating the advantageous female triploid (XXX) grass carp, *C. idella* or black carp, *Mylopharyngodon piceus* the main obstacles of marketing abroad such unique fish were caused by local legislations that blocked importation of these species. Introduction of black carp in the U.S. has been discouraged due to ecological concerns and its use in African countries as a biological control of bilharziasis lacked appropriate funding.

In this paper, chromosome-set manipulation activities carried out in Israel are separately presented for each research center. Among these centers only the YAFIT Laboratory is associated with a commercial fish breeding facility and fish farm. The other three institutions are public or national organizations: the Israeli Ministry of Agriculture, the Israel Oceanic and Limnological Research, and Bar-Ilan University (Table 1).

Aquaculture Research Station – Dor

Gynogenesis and polyploidy in Japanese (koi) carp (Cyprinus carpio). Exposing the zygote to shock is necessary to increase the incidence of diploidy or to induce polyploidy in the haploid embryonic genome. A series of studies were carried out to evaluate and optimize early and late application of heat- and cold-shocks in koi (Cherfas et al., 1990).

Dor scientists used the τ_0-parameter (Dettlaff and Dettlaff, 1961) to determine the initiation of shocking and obtained the highest yields of diploid meiotic or mitotic gynogeonotes by heat shocking when the τ_0-duration corresponded to the end of meiosis (second polar body retention – 2PB) or the first mitotic cleavage at metaphase and anaphase, e.g. 0.15-025 τ_0 or 1.5τ_0 after insemination, respectively (Cherfas et al., 1994a). In cold-shock experiments, the highest yields of meiotic and mitotic gynogenotes were obtained by applying the shock at 0.05-010τ_0 and 0.39-0.49τ_0, respectively (Cherfas et al., 1994b).

Spontaneous diploids were detected among hatched larvae in haploid controls. Such diploids may have occurred due to spontaneous diploidization of the maternal genome (Stanley, 1961; Cherfas et al., 1991) by non-disjunction occurring during the first or second meiotic division (Komen et al., 1991). The same phenomenon was detected in koi (Rothbard, 1991).

Triploid common carp. Triploidy in common carps was induced by heat-shocking normally fertilized carp eggs of the Dor-70 line. The triploidy was evaluated in larval stages by the number of nucleoli per nucleus or quantification of DNA in the nuclei of erythrocytes and in the majority of the fish, and by comparing sizes and shapes of erythrocytes and their nuclei to their respective diploid controls (Cherfas et al., 1994).

To assess growth potential of the triploid

Table 1. Chromosome-set manipulation activities in fish, as carried out in Israeli research centers (1980-2005).

Research center	*Chromosome-set manipulation activity*	*Current status*
Fish Culture Research Station, Israel Ministry of Agriculture, Dor	Gynogenesis in koi	Technology available
	Triploidy in common carp	Retarded growth of 3N fish
	Integration of gynogenesis and sex-reversal in common carp	Commercially applicable
YAFIT (R&D) Laboratory, Fish Breeding Center, Gan Shmuel	Gynogenesis and androgenesis in koi	Technology available
	Gynogenesis and polyploidy in black carp	Technology available
	Gynogenesis and polyploidy in grass carp	Technology available
	Gynogenesis in fantail goldfish	Technology available
	Computation of embryonic age in 9 species of fish	τ_0 Application proven
	Short term preservation of carp (koi) eggs	Application proven
	Integration of gynogenesis and sex-reversal in common carp	Commercially applicable
National Center for Mariculture, Israel	Gynogenesis in gilthead seabream	Technology available
Oceanographic and Limnological Research, Eilat	Allotriploidy in gilthead bream, using red seabream as sperm donor	Technology available
Fish Immunology and Genetics, Bar Ilan University	Gynogenesis and triploidy in tilapia	Technology available
	Androgenesis in tilapia	Technology available

and diploid common carp (*C. carpio*), experimental groups of fish were examined in communal ponds (fish groups, marked by different brandings, cultured in the same pond; see Wohlfarth and Moav, 1985). In general, triploids grew slower than diploids and triploid males grew slower than triploid females (Cherfas et al., 1994a).

Integration of gynogenesis and hormonal sex-inversion in common carp. Gynogenetic offspring (female monosex) of carp and koi were sex-inverted with methyl-testosterone (MT; 100 mg/kg food) and yielded high rates of neomales (40-95%). The success of MT-treatment was dependent on water management (recirculated versus running water) and

day of initiation of feeds containing hormones (27 versus 40 days post-hatching). Similar results were obtained for koi as well as for common carps (Gomelsky at al., 1994).

Gynogenetic females were sex-inverted with MT to produce neomales (XX-genotype; F_1-generation). Sperm of these neomales was used to fertilize eggs obtained from normal carp to produce monosex females (the F_2-generation) on large scale. The F_2 females were again sex-inverted using Fadrozole (aromatase inhibitor - AI). The efficiency of sex-inversion using feeds containing MT (40 and 100 mg/kg food) or AI (200 mg/kg food) was evaluated by the yield of neomales. The rate of neomales resulting from AI-treated fish was higher (58.6%) than for fish treated by MT (4.7-9.4%).

Growth performance of carp female-monosex offspring derived from neomales crossed with normal females was assessed for commercial fish culture. The growth of the females prior to sexual maturation was about 15% faster than of the males (Cherfas et al., 1996). These findings served as a significant stimulus to implement the technology and make it available for commercial aquaculture.

YAFIT (R&D) Lab

Pioneer work on ploidy manipulation in tilapia. The first attempts in Israel to induce chromosome-set manipulations in tilapia were carried out in the Gan Shmuel Fish Breeding Center, by Chourrout and Itskovich (1983). The determination of ploidy levels in different groups of fish was carried out by simple nuclear examination. Eggs of *Oreochromis niloticus* were fertilized with homologous sperm and heat-shocked for 2-7 min at 30.5-41.0°C, four minutes post-fertilization to retain the second polar body (2PB). High rates of triploid offspring were obtained that reached the feeding stage.

An interesting hybridization experiment was carried out involving a mouthbreeding female of *O. niloticus* and a male of the substrate spawner *Tilapia rendalli.* Although survival of embryos was low in this intergeneric hybridization, the yield of viable larvae increased dramatically when they were triploidized. It is possible that *O. niloticus* eggs, activated with *T. rendalli* sperm and shocked, generated not only triploids but also gynogenetic offspring of *O. niloticus*. This phenomenon was verified by karyotyping (Chourrout and Itskovich, 1983); the number of chromosomes in tilapia of normal fish and diploid gynogenotes was 44, but 66 in triploids.

The goal of these experiments was achieved with successful induction of gynogenesis in *O. niloticus* obtained from *O. niloticus* eggs inseminated with UV-irradiated sperm of red Taiwanese tilapia (probably an *O. mossambicus* hybrid derivative), then heat-shocked at 41-41.5°C for 3.5 min, starting the shock 5 min after fertilization. Non-shocked zygotes yielded only haploids that did not survive beyond the yolk-sac stage. The diploid gynogenotes were gray-pigmented and had the maternal phenotype, indicating successful induction of gynogenesis since the gray coloration of *O. niloticus* dominates the recessive red coloration of the Taiwanese fish. However, at present, *post factum*, we wonder whether these were truly gynogenotes or just normal hybrids.

Meiotic and mitotic gynogenesis in Japanese (koi) carp. Experiments aimed to induce mitotic gynogenesis (endomitosis) by late shock were carried out in the YAFIT laboratory to generate broodstock that could serve as parents for the second generation of gynogenotes, presumable clones (Rothbard, 1991). Koi eggs were inseminated with UV-irradiated sperm of wild-type (dominant) colored carp that served as a color marker. Carp of the Dor-70 line with the recessive gene for gold coloration (Wohlfarth et al., 1975) were excluded from these experiments. The dominant wild-type coloration allowed evaluation of successful irradiation of sperm. Survival of gynogenetic embryos from late-shocked eggs at the embryological age of $1.3\tau_0$ (32.5%) was higher than those shocked at $1.6\tau_0$ (14.7%). Spontaneous diploidization of the maternal genome was detected among non-shocked control embryos, as previously reported by Cherfas et al. (1991) and Komen et al. (1991).

Meiotic gynogenotes of the second gener-

ation of koi were produced by early heat (40°C/2 min) or pressure (ca. 7500 psi/2 min) shock from gametes of three 2-year-old females previously generated by late-shock induction. Survival of diploid gynogenetic larvae was significantly lower (2.1-7.6%) than in the respective fertilization controls (39.8-64.8%). The yield of gynogenotes from the three females was about 10,000 larvae. Although these should have been expected to be clones, with colors similar to their mothers' (tri-color), when they reached fry size (2-4 cm), the colors and color patterns were variable and not uniform. The color variation among these putative cloned fish suggests that other factors affect coloration. Presumably, a combined effect of genetic (polygenic inheritance), environmental, and physiological (hormonal) factors may change colors even in mature and large size fish.

Evaluation of the mitotic duration (Dettlaff and Dettlaff, 1961) in fish species by using the τ_0 parameter for timing ploidy manipulations is an important tool for ploidy manipulation, since it provides a dimensionless measure that incorporates the temperature effect on the developmental rate, relative to the initiation of shocking the developing zygotes. Since τ_0 is species-specific, this important criterion was defined for common carp, grass carp, and black carp and computerized for nine different fish species (Shelton and Rothbard, 1993; Rubinshtein and Rothbard, 1997).

Androgenesis in koi. There are two objectives for inducing androgenesis in koi: (a) generating YY-males capable of yielding exclusively Y-sperm and (b) preserving sperm of unique koi in a sperm bank (Lubzens et al., 1993).

Carp females of the Yugoslavian (Nasice) line were selected as egg donors for koi androgenesis. This strain, contrary to Dor-70 carp, does not carry a recessive gene for gold coloration (Wohlfarth et al., 1975) that interacts with the coloration of koi and therefore can serve as a color marker for the identification of androgenetic offspring.

The highest survival of androgenetic koi larvae resulted from common carp eggs irradiated with a UV-dose of 2500 J/m^2 and immersed in natural ovarian fluid to prevent post-activation stickiness. The inseminated eggs were heat-shocked (40°C for 2 min) during metaphase of the first mitotic division at the embryonic age of 1.5-1.7τ_0 (survival 5.4-7.8%). Most of the hatched larvae were malformed, probably due to increased homozygosity. Although the fry survived for several months, all eventually died. The last fish from the group died at 5-7 cm (Rothbard et al., 1999).

Gynogenesis in fantail goldfish. Attempts were made to induce gynogenesis in the redcap fantail, a variety of goldfish (*Carassius auratus*). Methods used for koi and common carp (heat-shock duration and level, τ_0, etc.), were successfully applied (unpubl. data). Since the goldfish does not become fully colored until the age of 2-3 months, we needed to find an effective genetic (phenotypic) marker to assess the success of gynogenesis at the earliest possible stage of development.

During the investigation it was observed that the single tail of the comet goldfish dominates over the fantail of the redcap and therefore it can serve as a reliable phenotypic marker. As a result of this finding, UV-irradiated sperm of comet was used to activate redcap eggs. The fantail characteristic could be clearly detected among the gynogenetic progeny after only a few days and well before the color normally develops in the larvae, indicating successful gynogenesis.

Integration of gynogenesis and sex inversion, female triploidy, and androgenesis in grass carp. Attempts were made in a series of experiments to produce meiotic (2PB) and mitotic gynogenotes, triploid females (XXX), and androgenetic (YY) grass carp (Fig. 1). Tetraploidy in grass carp was induced to generate broodfish capable of producing diploid gametes that, when coupled with haploid gametes, yield triploid offspring. Nevertheless, the preliminary experiment to induce tetraploidy yielded weak tetraploid larvae that did not survive beyond the larvae stage (7-10 days; Rothbard et al., 2000).

The τ_0-curve for grass carp (Shelton and Rothbard, 1993) served to define the precise

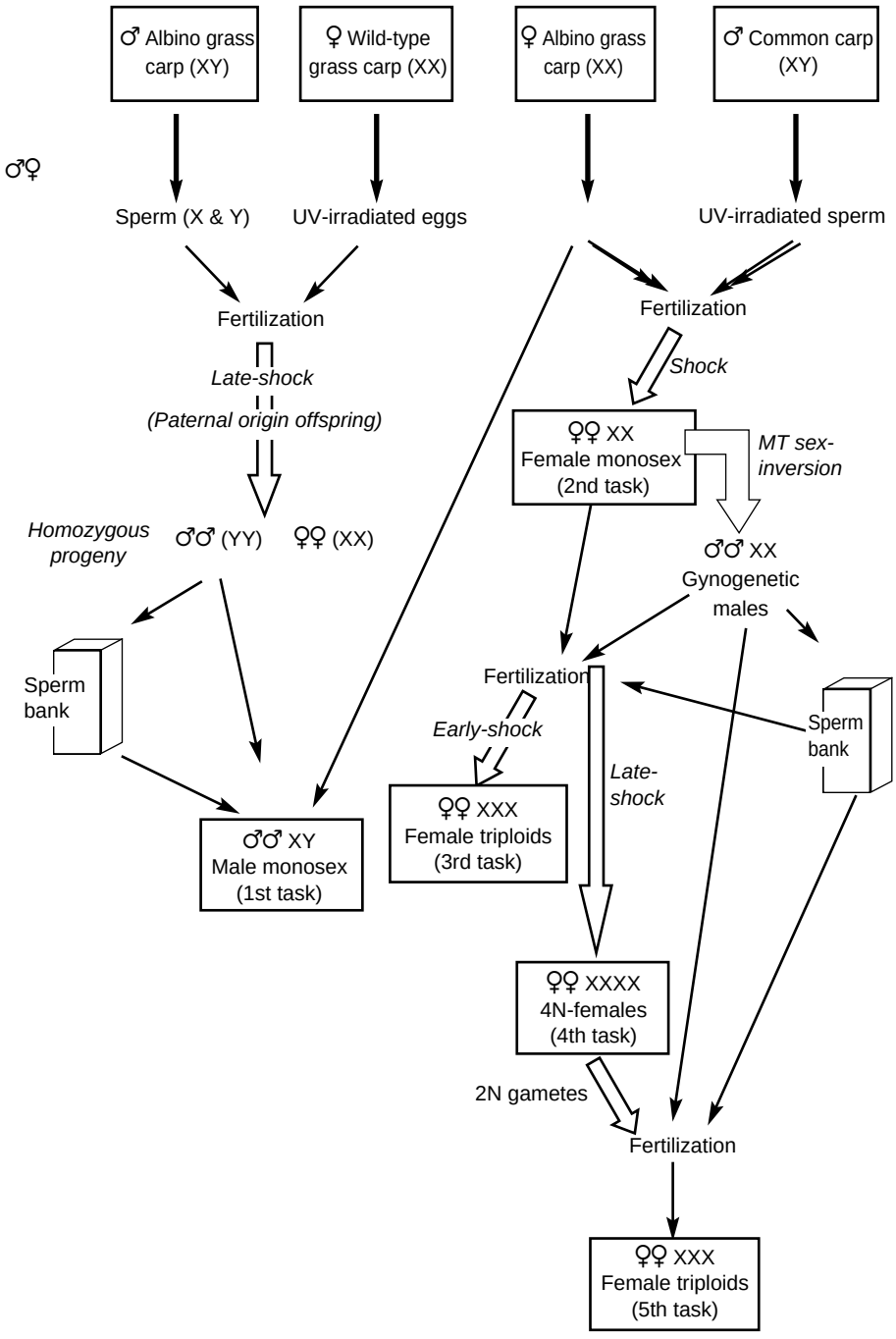

Fig. 1. Ploidy manipulations carried out in the YAFIT laboratory on grass carp (Rothbard et al., 2000).

timing for application of early and late shocks. Female albino grass carp were used as female broodstock to take advantage of the recessive color marker in ploidy manipulations. Cold (10°C/10-30 min), heat (40±0.5°C/2 min) and pressure (7000-9000 psi/35-120 sec) shocks were tested to induce the expected ploidy levels (Rothbard et al., 2000)

Ploidy levels of various groups were analyzed by fluorocytometric methods and are shown in Fig. 2. Tetraploid fish were successfully generated and confirmed by fluorocytometry, however, the fish did not survive beyond the larvae stage and therefore tetraploidy requires further investigation.

There is an advantage to producing triploid females (XXX). Unlike triploid males (YXX) that possess testicular fragments that occasionally produce a very low number of viable spermatozoa (Allen et al., 1986) and fertilization ability (Van Eenennaam et al., 1990), XXX-females are totally sterile, lacking gonads and exhibiting almost all-somatic development. Gynogenetic grass carp fingerlings (ca. 10 cm body length) were sex-inverted with MT-implants (Jensen et al., 1983; Shelton, 1986) and grown to maturity as neomales that produce exclusively X-bearing spermatozoa. Viable sperm from 2-year-old neomales was easily stripped and used to fertilize eggs of XX females to produce sterile

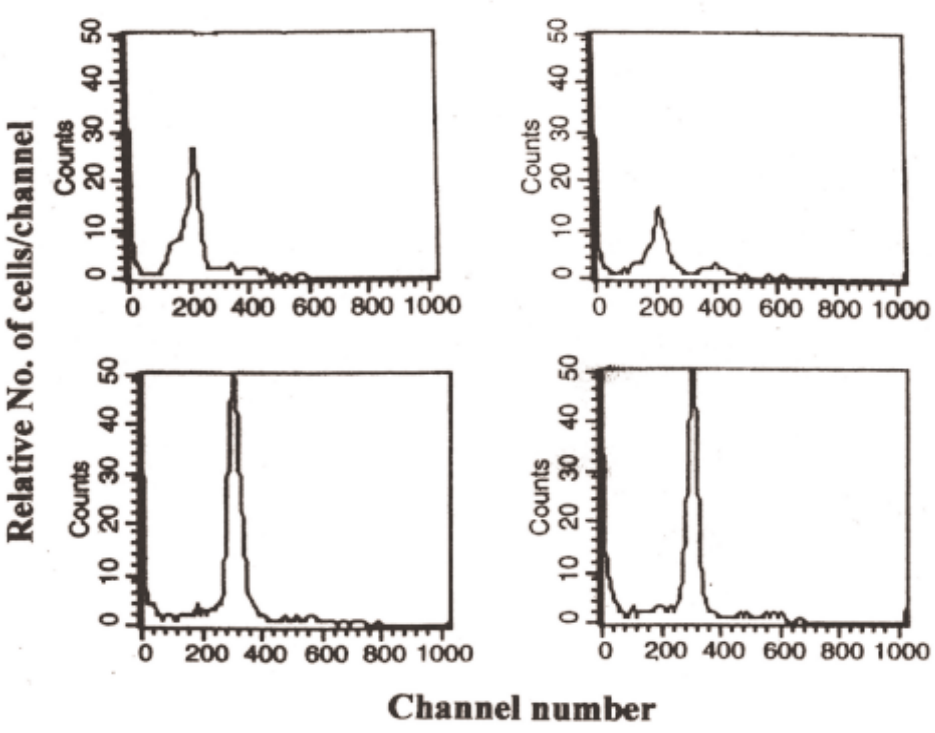

Fig. 2. Fluorocytometric analysis of diploid and triploid grass carp erythrocytes, carried out in YAFIT laboratory: (a) diploid larva, (b) triploid fingerling, (c) diploid larva, and (d) triploid fingerling (Rothbard et al., 2000).

XXX-triploid females; some sperm was cryopreserved in a sperm bank.

Gynogenesis and female triploidy in black carp. The first attempt to induce meiotic gynogenesis in black carp ova (2N = 48) was performed using UV-irradiated sperm of heterologous species, koi and goldfish, which possess a different number of chromosomes (2N = 100). Fertilization of black carp eggs with such sperm results in non-viable offspring (Rothbard and Shelton, 1993). However, exposure of activated eggs to heat or pressure shocks restored diploidy. Another set of chromosome-set manipulations followed the first successful gynogenetic induction (Rothbard et al., 1997). In this follow-on study, the highest survival of gynogenetic larvae was obtained when eggs were early heat-shocked (41±1°C/1 min) or pressure-shocked (7500-7600 psi/1.5 min) at the embryonic age of 0.08-0.16τ_0.

Triploid black carp were obtained from normally fertilized eggs that were early (0.15-0.16τ_0) heat or pressure shocked to retain the second polar body. The ploidy level of diploids and triploids was evaluated by fluorocytometry (Fig. 3).

Integration of gynogenesis and hormonal sex-reversal in common carp. Meiotic (2PB) gynogenesis was induced in the Yugoslavian strain of common carp eggs. UV-irradiated sperm of comet goldfish provided a genetic marker, i.e., the scale coverage of the goldfish, and was used to inseminate common carp eggs. The activated eggs were cold-shocked (ca. 5°C) for 45-50 min to diploidize the haploid zygote. The amphimictic hybrids (intact carp eggs fertilized with intact goldfish sperm) served as a scaled control (Fig. 4).

Gynogenetic carp fry (1.5-2 g avg body wt) were fed for two months on feed that contained Fadrozole, an aromatase inhibitor (AI; CGS 16949A, Novartis Pharma AG, Switzerland), at 200 mg AI/kg feed (Rothbard et al., unpubl. data). The AI-treated fish (neomales) continued to grow to about 20-50 g when they were examined for sperm by gentle pressure on the abdomen and the sperm was microscopically examined for motility. About 50% of the fish (200-250 fish) released drops

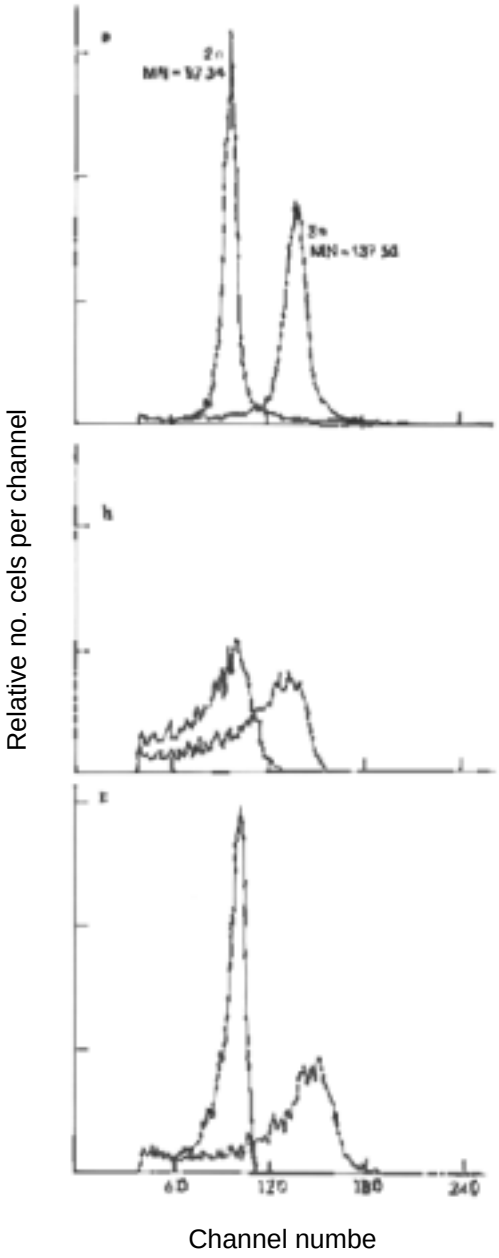

Fig. 3. Fluorocytometric analysis of black carp cells from (a) hatchlings, (b) young fry, and (c) erythrocytes. MN = mean channel position calibrated cells taken from homologous controls, 2n = diploids, 3n = triploids (Rothbard et al., 1997).

Fig. 4. Amphimictic hybrids of common carp x goldfish. The fish served as a control in experiments in which gynogenesis was induced by insemination of common carp eggs with goldfish UV-irradiated sperm.

of viable and motile sperm. Sperm samples were cryopreserved and stored in a sperm bank; some was used for commercial production of all-female carp populations. The other fish were discarded due to body malformations caused by the haploid syndrome occurring at gynogenesis (Fig. 5). At present, about 1.5 million putative female offspring of three Dor-70 females are being nursed in earthen ponds. When the fish attained the size appropriate for sexing (20-50 g), a sample of 30 fish was examined to verify the monosex population. All fish in the sample were female.

National Center for Mariculture (IOLR) – Eilat

Gynogenesis and allotriploidy in gilthead seabream (Sparus aurata). The aim of ploidy investigation in gilthead seabream was to generate inbred lines that, when crossed with fish from different and distant populations, would have improved growth due to the advantage of hybrid vigor.

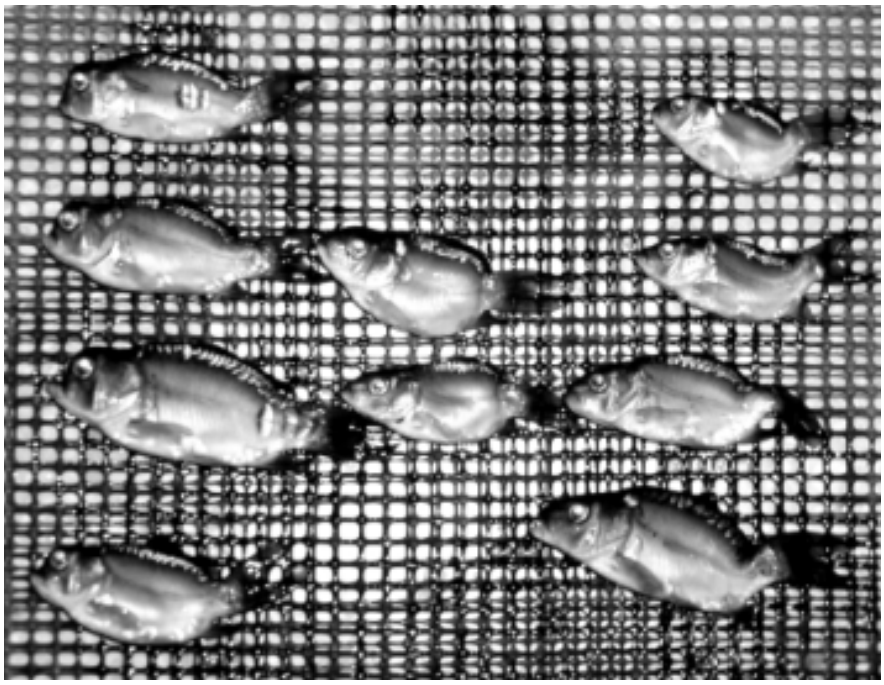

Fig. 5. Malformed carp fry resulting from haploid syndrome or insufficient application of physical parameters at ploidy manipulation (Rothbard, unpubl.)

UV-treated sperm of red seabream, *Pagrus major*, served as genetic marker. The sperm was used to activate gilthead seabream eggs and produce gynogenetic diploids. Ploidy levels of offspring derived from various chromosome-set manipulations (haploids, gynogenetic or amphimictic diploids, allotriploids; 24, 48, 72 chromosomes, respectively) were identified by the differences in the structures of metacentric and sub-metacentric chromosomes of these two heterologous species and by karyotyping (Gorshkov et al., 1998).

Ploidy manipulations in the European seabass, Dicentrarchus labrax. European seabass is a commercially important marine fish, endemic to the Mediterranean Sea basin. Extended research on chromosome-set manipulations has been conducted with this species. The long-term final target of investigation was to generate all-female seabass populations, characterized with advantageous growth performance.

Gynogenetic seabass were obtained by exposing eggs to early heat-shock (35°C applied 3-5 min post fertilization). UV-irradiated homologous sperm, karyologically examined for success of irradiation, was used to activate eggs. Non-shocked eggs developed as haploid embryos that did not survive while shocked eggs yielded gynogenetic fish. Since there are structural differences between male and female chromosomes, the success of manipulation was confirmed by karyological examination (Gorshkova et al., 1995, 1996).

The efficiency of triploid seabass production was rather high; the rate of triploid fish exceeded 80%. Exposure of seabass eggs fertilized with homologous sperm to heat-shock, similar to the shock used to induce gynogenesis, induced the triploidy. The GSI (gonado-somatic index) of 2-3 year-old triploid females was much lower than of the diploid fish. Moreover, preliminary data indicated that the triploids were characterized by retarded growth when compared to their respective diploid control (Gorshkova et al., 1996).

Laboratory of Fish Immunology and Genetics – Bar Ilan University

Gynogenesis in tilapia, a model of tilapia sex determination. The Hertwig effect was examined to define the optimal UV dose effective for inactivation of tilapia sperm, which can serve for inducing gynogenesis in tilapia (Don and Avtalion, 1988a). UV-irradiated sperm of Nile tilapia, *O. niloticus*, characterized by dominant genetic markers (serum esterase and tail stripes), was used to inseminate *O. aureus* eggs. The gynogenetic offspring suffered from body malformations and very low survival. Higher embryo survival was obtained from the F_2 rather than the F_1 generation (0.36% and 3.6%, respectively). This phenomenon was explained as a result of reduced levels of recombination in the second gynogenetic generation and lower expression of lethal and defective genes. The experimental conditions required to induce ploidy and gynogenesis in *O. niloticus* and *O. aureus* are reported by Don and Avtalion (1988b) and by Shirak et al. (1998).

Triploidy and tetraploidy in tilapia. Triploidy was induced in two tilapia species, *O. niloticus* and *O. aureus*, by exposing fertilized eggs to early heat (about 40°C/3.5-4 min) or cold (11°C/60 min) shock. Higher survival of *O. aureus* triploids (60% vs. 13%) was attributed to the genetic contribution of the maternal inheritance (Don and Avtalion, 1988c). Better results were obtained when the eggs were cold-shocked (50%-60%), probably due to the possibility that cold shock interferes with two distinct meiotic cell divisions. Nevertheless, the authors noticed that cold and heat shock are equally effective in inducing triploidy in *O. aureus*.

In another study, diploid (normal) embryos of *O. aureus*, incubated in 25-26°C, were cold-shocked at the zygotic age of 92 min and during zygotic age intervals lasting between 80 to 104 min. Shocking at the zygotic age of 92 min resulted in higher yields of tetraploids. Ploidy of the derived embryos was assessed by karyotyping and by orange stained FACS (fluorescent activated cell sorter) analysis of the adult fish (Don and Avtalion, 1988d).

Degree of inbreeding in gynogenetic tilapia. The rate of inbreeding in tilapia produced by gynogenesis was compared to amphimictic offspring. Fish of the first generation of gynogenetic *O. aureus* were compared to their female parents and to *O. niloticus* by examining immune rejection of transplanted scale grafts (Avtalion et al., 1988). No rejection of transplanted scales from *O. niloticus* parents was observed among the gynogenetic *O. niloticus* offspring. A similar response to skin allografts was reported in common carp by Komen et al. (1990). However, acute rejection of transplanted scales was observed among allogeneic *O. aureus*, probably due to using fish from strongly selected inbred lines. Nevertheless, the results were not unequivocal for other examined groups and further investigation of this promising tool for measuring inbreeding in tilapia was suggested.

Summary and Conclusions

The progression of chromosome-set manipulation practice in Israeli aquaculture centers is remarkably impressive. Successful technologies have been developed for ten fish species and varieties, including two commercially important marine species. Nevertheless, the practical and commercial impact of ploidy manipulation research, carried out in four Israeli laboratories, is quite limited. One notable exception is the production of common carp broodstock (YAFIT Laboratory and Dor Station) that yield all-female populations on a commercial scale. In this technology, fish obtained by gynogenesis are used to generate neomales with the female genotype (XX-sex determination mechanism).

Production of common carp neomales has been dramatically facilitated by introduction of Fadrozole, an aromatase inhibitor, that very successfully and efficiently replaced the formerly used MT in androgenic sex-inversion.

Although ploidy manipulation of the two marine species, gilthead seabream and European seabass, has promising future application, it must still be proven and extended to fill missing gaps, e.g. field experimentation as well as progeny testing.

Much work has been invested in development of technology for chromosome-set manipulations in tilapia. Two of the most important tilapia species worldwide, *O. aureus* and *O. niloticus*, were selected for investigation. The basic research aimed at understanding the intriguing puzzle of sex-determination in tilapias. Ploidy manipulation technology integrated with hormone treatment may permit generation of YY-males (in *O. niloticus*) or ZZ-males and ZZ-neofemales (*O. aureus* neofemales were reported by Rosenstein and Hulata, 1993) that, in appropriate mating combinations, may yield the desired all-male populations. Nevertheless, results have not yet been commercially implemented. Most commercially applied all-male production of tilapias exclusively uses androgen sex-inversed technology, however, concerns about human consumption of hormone-treated fish has caused increasing constraints. Using genetically modified fish could provide a solution if the basis for sex determination in fishes can be resolved. The technology was extended and hormonal sex-inversion was implemented on one Israeli fish farms (Nir David Fish Breeding Farm) to produce *O. aureus* neofemales with the male (ZZ) genotype. Such females can yield male-monosex populations when mated with amphimictic males (Lahav, 1993).

Since 1987, intensive research has been carried out on chromosome-set manipulations with the Japanese ornamental (koi) carp. The aim of these investigations was to produce koi clones through two-step gynogenesis. During the first stage, gynogenetic and totally homozygous koi were obtained by induction of mitotic (late shock) gynogenesis. The resulting gynogenotes served as ancestors for a second generation of meiotic gynogenotes. The offspring obtained in the second gynogenetic generation are expected to be clones due to homozygosity and the absence of recombination and crossing-over at meiosis. Nevertheless, in spite of success in gynogenesis, the colors of offspring indicated that there are other factors that are difficult to control, e.g. possible genotype-environment interaction or polygenic inheritance which probably affects uniformity of coloration and color patterns of the derived progenies (Rothbard and Wohlfarth, 1995). At pre-

sent, it seems that further progress to improve coloration of koi can be achieved only with the assistance of DNA markers. The onset of such long-term research was recently reported by David et al. (2001, 2004).

Generation of unique fish during the process of ploidy manipulation such as koi, common carp, and grass carp neomales or the need to preserve unique genetic traits was a major stimulus for establishing a fish sperm bank. Such a bank allows long-term preservation of gametes with unique characteristics that have been consolidated or developed through genetic manipulation (Lubzens et al., 1993). Sperm of various lines of koi, carp, and grass carp have already been stored in the bank for possible prospective use.

Possession of albino grass carp and the option to use sperm of heterologous species have facilitated ploidy manipulations with both grass and black carp. Male triploids (YXX-triploids) can develop gonads with testicular fragments (Allen et al., 1986) and viable spermatozoa and are therefore considered only partially sterile. Therefore, a method to produce totally sterile female triploids (XXX-triploids) was developed by integrating gynogenesis and MT sex-inversion to produce grass or black carp neomales as broodfish. Male triploids of grass carp have been widely used in the USA for many years as a biocontrol of water vegetation (Allen and Wattendorf, 1987). In spite of being extensively documented as a commercially important species and component of biological control, export of these advantageous female triploids was halted by strict governmental legislation or lack of funding.

Although considerable research has been completed, only a small amount of the developed technology has been commercially implemented. It usually takes 10-20 years from being experimentally established until applications are adopted and practiced by industry (Hulata, 2001).

Acknowledgement

I wish to thank Prof. W.L. Shelton (Oklahoma State University, Norman, OK, USA) for his valuable comments and suggestions in preparation of this manuscript.

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