LONG TERM EFFECTS OF MASCULINIZING TREATMENTS ON THE REPRODUCTIVE CHARACTERISTICS OF GREY MULLET (*MUGIL CEPHALUS*)

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Introduction:

The grey mullet, *Mugil cephalus,* are fished and farmed world-wide. To avoid the continuous pressure on the wild populations, we developed at IOLR-NCM successful breeding [1] and larval rearing protocols for captive mullets, along with developing formulated growout feeds for their rearing to market size. Following a successful completion of this research, improved economics of production is expected by culturing all-female mullet populations, largely due to their highly prized roe used for preparing a seafood delicacy called "Karasumi". To achieve this goal, the current study has adopted the indirect feminization strategy [2], involving the masculinization of genotypic females, and crosses of the produced neomales with normal females, to produce a female monosex mullet population. Previous studies have demonstrated successful sex reversals in mullet using both an androgen to masculinize [3] and an oestrogen to feminize [4] the fish. However, there was no information whether the masculinized fish turned to be functional phenotypic males at maturation. Therefore, our specific objectives were to follow the sex-reversed fish to sexual maturity and to evaluate their phenotypic sexual stability and fertility.

Methods:

Experimental Fish - Hatchery produced mullet fry at our facility (IOLR-NCM) were maintained in 1- 5 m^3 tanks, supplied with ambient (Gulf of Eilat, Red Sea) seawater at 40 ppt salinity and subjected to natural fluctuations of photoperiod, light and temperature. Fish were fed daily at the rate of 1.5% of their body weight using a 35% crude protein and 7.2% lipid diet, according to our (IOLR-NCM) feed formulation and feeding regime.

Induced masculinization - A masculinized phenotype was obtained by exposing for 4 months, mullet fry, at 3 age categories (3, 6 and 9 month old), to food supplemented with either: (i) methyltestosterone (MT; 15 or 10 mg MT/Kg of food) or (ii) Fadrozole (100 mg/Kg food). In order to study the phenotypic sexual stability, the 6 month old group that obtained the primary treatment of 15 mg MT/Kg food was divided randomly into two groups: one received repeated MT exposure via a slow release vehicle (administered via ethylene-vinyl acetate copolymer [EVAc] implants; 5 mg MT/pellet) at 2, 3 and 4 years after the primary treatment. The second group was used as untreated controls, which obtained only the primary treatment (see above). Sex was determined by vitellogenin dot blot analysis and/or gonadal biopsies [1]. Presence of milt in male mullets was checked by applying gentle abdominal pressure and a sample of milt was collected to evaluate sperm motility, morphology and spermatocrit.

Results and discussion:

As in wild stocks, the sex ratio among control mullet groups did not differ significantly ($P > 0.05$) from the expected 1:1 male:female ratio. These results rule out the occurrence of skewed sex ratios due to culture conditions, and are consistent with those of previous studies carried out in Taiwan [3, 5] and North Carolina [6].The results of the masculinizing studies further define, in mullets, the period of 6 to 9 month of age, as a labile phase when the differentiating gonads are most susceptible to androgens.

The most potent treatment [MT-6] gave rise to 100 % males upon the completion of sexual differentiation. Nevertheless, at sexual maturity (3 years after treatment) markedly lower male percentages (70%) were observed in this group, suggesting that the grey mullet can spontaneously sex reverse. Interestingly, higher male percentages (90%) were detected among retreated fish boosted with MT containing EVAc implants. The milt produced by the latter fish revealed characteristics (i.e. sperm count, motility and morphology) resembling those found for untreated males.

Conclusion:

The grey mullet appears to have high sexual plasticity also away from the sex-differentiation period, which is atypical to most other gonochoric fish that exhibit fixed sexuality once sex differentiation is being completed. Similar sexual plasticity was recently documented also in trout [7], which attests to a spontaneous sex reversal and stresses the need to preserve functional phenotypic sex by repeated treatments. Besides their aquaculture applicability, our results propose the grey mullet as an important model for further studying germ cells plasticity in fish.

References:

[1]AIZEN, J., MEIRI, I., TZCHORI, I., LEVAVI-SIVAN, B., ROSENFELD, H. 2005. Enhancing spawning in the grey mullet (*Mugil cephalus*) by

removal of dopaminergic inhibition. Gen. Comp. Endocrinol., 142: 212-221.

[2]PIFERRER, F. 2001. Endocrine sex control strategies for the feminization of teleost fish. Aquaculture, 197:229-281.

[3]CHANG, C.F., HUNG, C.Y., CHIANG, M.C., LAN, S.C. 1999. The concentrations of plasma sex steroids and gonadal aromatase during controlled sex differentiation in grey mullet, *Mugil cephalus*. Aquaculture, 177:37-45

[4]CHANG, C.F., LAN, S.C., PAN, B.S. 1995. Feed administration of estradiol-17 beta stimulates female differentiation in juvenile grey mullet *Mugil cephalus*. Zool. Studies, 34:257-264.

[5]CHANG, C.F., LAN, S.C., CHOU, H.Y.1995. Gonadal histology and plasma sex steroids during sex differentiation in grey mullet, *Mugil cephalus*. J. Exp. Zool., 272:395-406

[6]BICHY, J.B. 2004. A life history assessment on the reproduction and growth of striped mullet, *Mugil cephalus*, in North Carolina, MA thesis, North Carolina State University.

[7]YOSHIZAKI, G., ICHIKAWA, M., HAYASHI, M., IWASAKI, Y., MIWA, M., SHIKINA, S., OKUTSU, T. 2010. Sexual plasticity of ovarian germ cells in rainbow trout. Development, 137(8):1227-30.