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Masculinization of GYMNOCORYMBUS TERNETZI (Boulenger) Using 17 α -Methyl Testosterone with Continuous Immersion

KEYWORDS: Masculinization - 17 α - MT - continuous immersion - gonadal squash - G.ternetzi

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ABSTRACT *In Gymnocorymbus ternetzi , 100% masculinization had been induced successfully, for the first time , using continuous immersion techniques with 17 α - Methyl testosterone. Control population had a highly skewed sex-ratio in favour of males (59%), possibly due to temperature induced / based natural sex reversal. Intersexes were also observed in the control stock. For 10µg/l (single dose) of continuous immersion of 5 –day old posthatchlings with 17 α - Methyltestosterone resulted in cent percent males with survival rate of 42% However, a strict dose -dependent mortality was not observed. Super.-optimal doses of 50, 100µg/l of 17 α -MT demonstrated paradoxical feminization. However, a strict dose -dependent mortality was not observed. Intersexes were observed in the hormone exposed lots at both sub-optimal and super-optimal doses. Sexes were identified through gonadal squash.*

INTRODUCTION

Artificial sex reversal usually involves artificial manipulations of the embryonic sex differentiation of a normally gonochoristic species, resulting in a phenotypic sex dishormonious with the genotypic sex (Chan & Yeung, 1983). Sex reversal, the transformation of an individual from one sex to another, is defined by Atz (1964) as change "from the possession of recognizable ovarian tissue to that of testicular tissue or vice versa."

In 1983, Yamazaki claimed that functional endocrine sex reversal has been successfully achieved in 15 gonochoristic species (5 families) using one or the other of 14 (8 androgens; 6 estrogens) steroids. But at present, treatment protocols are available for 47 species (15 families) of gonochores (34 species; 9 families) and hermaphrodites using one of the 31 (16 androgens; 15 estrogens) steroids (Pandian & Sheela, 1995). However, characins remain unventured with regard to masculinization with hormone treatment , hence the present attempt.

MATERIAL AND METHODS

Experimental fish

Amidst the bewildering varieties of ornamental fishes, a vivacious, active, prized little fish, Gymnocorymbus ternetzi (Boulenger) , commonly known as black or widow tetra, belonging to family Characidae, an almost non-exploited family in the context of hormonal sex reversal, has been selected as the candidate species in the present study, aiming at the production of sex reversed populations.

Collection and maintenance of fish

G.ternetzi, obtained in their immature stage (30-45 days old), from local private ornamental fish dealers, were stocked in outdoor concrete tanks till they attained maturity. Later, they were transferred to indoor glass aquaria and maintained at 28 ± 1°C and 14L: 10D photothermal cycle. One week prior to breeding, sexes were maintained separately as it may considerably enhance the willingness to breed, besides avoiding breeding on their own without our eye on it.

Breeding in ornamental fish farm

Breeding was usually carried out in larger cement tanks of 200 - 700 l capacity. Females and males in the ratio of 5-6 to 10-12 were introduced into the tanks. Plants (Ceratophyllum sp.) were also put in. Spawning occurred in the morning, next day, and the parents were immediately removed. Three days after spawning, hatchlings started to swim freely.

Hormone administration

To ensure sex reversal, immersion method was adopted (Pandian,1993). For treatment, a stock solution of hormone was prepared by dissolving the steroid (Sigma, USA)-17a-Methyltestosterone (17a - MT), a synthetic hormone mostly preferred for achieving masculinization (Pandian & Sheela, 1995) - in an appropriate solvent (ethanol) at a concentration of 1mg/ml. The stock solution was then added to the rearing water to achieve the desired concentration and experiments were done. Controls with neither hormone nor solvent were run side by side.

Five day old post hatchlings obtained using 6-10 females and 12-20 males, were pooled and from that lot, required number of post hatchlings were used for hormone treatment. Feeding regimes were similar to farm practices.

In continuous immersion method , hormone dose was added to the same water and water was not changed here for 4-6 weeks.

Squash preparation

Juvenile fish (75 DAH) were sexed by examining the squash preparation of the gonad (Guerrero & Shelton, 1974). After displacing the viscera, a few drops of Carnoy's fluid were applied to the gonad, and a fraction of it was taken out. The gonad was placed on top of a clean slide and a few drops of acetic orcein were added before squashing with a cover slip. The mounted tissue was examined under a microscope. Females were identified by the presence of developing oocytes with lightly stained nucleus. Spermatocytes were clearly visible in males. Intersexes were identified by the presence of both male and female tissues in the same gonad. Squash preparations were photographed at 20 and 40 X magnification.

STATISTICAL ANALYSES

Sex ratio differences were treated with Chi-square (c^2) test , to find out the differences at various 'P' levels.

RESULTS

Sex ratio in control

The sex ratio in control was highly skewed in favour of males. Intersex were seen in the control stock and the proportion of females was found to be very low. The sexes, female : intersex : male were distributed in the ratio of 0.29 : 0.12: 0.59 in control.

Masculinization using 17α-Methyltestosterone (17α-MT)

Data on the effect of 17α-MT on the percentage of mascu-

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linization with single dose of continuous immersion for achieving 100% masculinization are given in Table 1 . A single dose of 10 μg/l in the continuous immersion experiments was significantly effective (P<0.001) in producing cent percent males, with a survival of 42%, which was better than the % survival rate of both the optimal single short term immersion experiment (22%) and control (35%). With increase in dose (50 & 100 μg/l) the % of males also increased as a result of paradoxical feminization and intersexes were observed at a dose of 100 μg/l. Sterile fish were however not observed at any dose and highest mortality (73%) was seen at 100μg/l.

Identification of sex

Sexes were identified based on the squash preparation of gonads. The spermatocytes and oocytes intermittently present in testicular tissue could be seen with intersexes

DISCUSSION

A skewed sex ratio in favour of males and the presence of intersexes in the control stock make the present study quite interesting. Occurrence of enhanced percentage of males in the population may be due to natural sex reversal . Natural sex reversal may be induced by extrinisic factors. The occurrence of various forms of Environmental Sex Determination (ESD) among fishes (temperature –, behaviour –, salinity - , light - , water quality - , pH – or nutrition – dependent sex determination) have been dealt in detail in a number of reviews (Chan & Yeung, 1983; Shapiro, 1990; Francis, 1992 , Pandian & Koteeswaran, 1999).

As with G.ternetzi ,other fish species such as the guppy, which have normally two distinct sexes and presumably a well defined genetic mechanism of sex determination, occasional hermaphrodites have been reported (Spurway, 1957) which is also the case with the common carp (Kossman, 1971).

In the present study, with continuous immersion using 17a-Methyltestosterone effective masculinization in the range of 55-100% has been achieved , which is in line with other species like O.mossambicus (Varadaraj & Pandian, 1987), Oncorhynchus kisutch (Piferrer et al., 1994), O. rhodurus (Nakamura, 1994) and Betta splendens (Kavumpurath, 1991). Intersexes as in the present study have also been reported to occur in other species due to steroid treatment at sub or superoptimal doses of hormone (Varadaraj, 1990; Okako & Phelps, 1995). Interestingly, paradoxical feminization was observed in the present study at superoptimal doses of 17 a-Methyltestosterone. Studies suggest that feminization by androgens might be the result of aromatase enzyme which converts androgens to estrogenic compounds.

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Table-1 : Effect of 17α-Methyltestosterone (17-α MT) administration through continuous immersion on the 5th day post hatchlings of G.ternetzi

S.No.	Dose (mg/l)	Initial No.	Survival % (at 75Days)	No. sexed	Sex distribution		
					F	I	M
1. Single dose							
(i)	10	100	42	20	-	-	1.0 [a]
(ii)	50	100	42	20	0.20	-	0.80 [a]
(iii)	100	100	27	13	0.46	0.15	0.39 [c]
II Control (average of 2 sets of each 100 hatchlings)							
		100	35	17	0.29	0.12	0.59

F- Female M – Male I – Intersex
a – P<0.001 b – P<0.005 C – P<0.05

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