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Masculinization of Gymnocorymbus Ternetzi (Boulenger) Using Short Term Immersion of 17 α -Methyl Testosterone

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

*** B.Uma**

Department of Zoology, Bharathi Women's College, Chennai –600108, Tamil Nadu, India.
* Corresponding Author

ABSTRACT *Immersion of fry of Gymnocorymbus ternetzi for short term in 17 α - Methyl testosterone for masculinization has been tried in the present study. 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. A single optimum dose of 400 µg/l of 17α-Methyltestosterone (17α-MT) for 4 hours through immersion of 5 day old posthatchlings induced 100% masculinization, with survival of 22%. However, a strict dose -dependent mortality was not observed. Super.-optimal doses [600, 800 µg/l for 4 hours] of 17 α -MT demonstrated paradoxical feminization. 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." At present, treatment protocols are available for 47 species of gonochores and hermaphrodites using one of the 31 steroids (Pandian & Sheela, 1995). However, characins remain unventured with regard to masculinization with hormone treatment , hence the present attempt.

MATERIAL AND METHODS

Experimental fish

Gymnocorymbus ternetzi (Boulenger) , commonly known as black or widow tetra, belonging to family Characidae, has been selected as the candidate species in the present study.

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. Five day old posthatchlings obtained using 6-10 females and 12-20 males, were pooled and from that lot, required number of posthatchlings were used for hormone treatment. For treatment, a stock solution of hormone was prepared by dissolving the steroid (Sigma, USA)-17α-Methyltestosterone (17α - MT), a synthetic hormone mostly preferred for achieving masculinization (Pandian & Sheela, 1995) - in an appropriate solvent (ethanol) at a concentration of 1µg/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.
In short term immersion experiments, after exposure for a definite period, the fry were transferred back to rearing tanks. Feeding regimes were similar to farm practices.

Squash preparation

Juvenile fish (75 DAH) were sexed by examining the squash preparation of the gonad (Guerrero & Shelton, 1974) under a microscope. 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 masculinization with the optimum dose for achieving 100% masculinization are given in Table 1 .Single short term immersion of 17α -MT for 4 hours at doses 200, 600, 800 µg/l was ineffective in producing an all-male population of G. ternetzi. However, an optimum dose of 400 µg/l was significantly ($P<0.001$) effective in producing 100% males and thus this characid is amenable for cent percent masculinization. At a dose of 1000 µg/l, fry never survived beyond a week.

While the optimum dose (400 µg/l) resulted in 22% survival on the 75th day after hatching, a dose of 800 µg/l also resulted in 21% survival. At doses 200 and 600 µg/l the survival rates were higher, 41& 46% respectively, than control (35%) . Thus a strict dose dependent mortality was not observed as an optimum dose of 400 µg/l had resulted in poor survival than with a dose of 600µg/l.

With an increase in dose (600 and 800 µg/l) the percentage of females also increased, reflecting clearly the aromatase activity upon Methyltestosterone (MT), resulting in paradoxical feminization . Intersexes obtained at lower doses of MT

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(200 µg/l) exposure had demonstarted the ineffectiveness of the dose used in masculinization, while the same observed at 600 µg/l, showed the incomplete paradoxical feminization.

Identification of sex

Sexes were identified based on the squash preparation of gonads. 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. The spermatocytes and oocytes intermittently present in testicular tissue can be clearly seen in the 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. (Pandian & Koteeswaran, 1998).

The best known example of temperature influenced sex reversal in fishes is reported in a member of the family Atherinidae, the Atlantic silverside, Menidia menidia (Strussman & Patino, 1995). Thus , in the present case with G.ternetzi also such temperature influenced sex reversal would have played a major role in the occurrence of reduced percentage of females as the rearing temperature has been 28 ± 1°C in addition to other unknown factors.

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). Further, it has also been suggested that the absence of a dual origin (lack of medullary tissue) of the gonad may also account for the more wide spread occurrence of hermaphroditism / intersexuality among teleosts (Nagahama, 1983).

In the present study, cent percent masculinization has been achieved with a single short term immersion in 17α-Methyltestosterone with the survival of 22 %. In a similar way, either with single or discrete or continuous immersion using 17α-Methyltestosterone effective masculinization in the range of 55-100% has been achieved with other species like O.mossambicus (Varadaraj & Pandian, 1987), Oncorhynchus kisutch (Piferrer et al., 1994), O. rhodurus (Nakamura, 1994), Betta splendens (Kavumpurath, 1991) and Clarias gariepinus (Hurk et al., 1989). High mortalities after the treatment with synthetic steroids have been reported for some species like S.trutta (Ashby, 1957) and S.gairdneri (Sower et al., 1983). It is well known that high doses of steroids inhibit liver and kidney functions of higher vertebrates (Serra et al., 1983).

Intersexes as in the present study (although observed in control stock, a higher percentage of it has been observed in the treated stock) 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). Appearance of intersexes may be species specific or in some cases may even be strain specific. Interestingly, increased percentage of females are observed in the present study at superoptimal doses of 17 α-Methyltestosterone. Similar paradoxical feminization has also been observed in various species of fish after treatment with methyltestosterone, such as Tilapia mossambica (Nakamura, 1975), Poecilia reticulata (Takahashi, 1977), rainbow trout (Solar & Donaldson, 1985), O.kisutch (Piferrer et al., 1994) and with mibolerone in O.mossambicus (Nandeesha et al., 1990). These studies suggest that feminization by androgens might be the result of aromatase enzyme which converts androgens to estrogenic compounds. Piferrer and Donaldson (1991) have cautioned that aromatization to estrogen does not completely exclude the possibility of an inhibition of the synthesis of endogenous androgen as a consequence of the synthetic androgen treatment, both processes may be occurring simultaneously.

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Table-1 : Effect of 17α-Methyltestosterone (17-α MT) administration through rearing water on the 5th day post hatchlings of *G.ternetzi* with single short term immersion (4hrs)

S.No.	Dose (µg/l)	Initial No.	Survival % (at 75 Days)	No. sexed	Sex distribution		
					F	I	M
(i)	200	100	41	20	0.30	0.15	0.55
(ii)	400	100	22	12	-	-	1.0 [a]
(iii)	600	100	46	22	0.36	0.28	0.36 [b]
(iv)	800	100	21	10	0.40	-	0.60 [b]
(v)	1000	100	-	-	-	-	-
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

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