RESEARCH PAPER | Zoology | Volume : 4 | Issue : 4 | Apr 2014 | ISSN - 2249-555X

Feminization of GYMNOCORYMBUS TERNETZI (BOULENGER) using Short Term Immersion with Estradiol –17 β

KEYWORDS: Feminization - Estradiol -17β- short term immersion - gonadal squash - G.ternetzi

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ABSTRACT *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. With Estradiol -17β, a single short term immersion of 600 µg/l for 7 hours yielded 61% females, while 600 µg/l for 10 hours resulted in 71% females. Among the two, treatment for 7 hours resulted in better survival (36%) than for 10 hours (11%) and thus recommended for adoption. Intersexes were observed in the hormone exposed lots at both sub-optimal and super-optimal doses. Sexes were identified through gonadal squash.*

INTRODUCTION

Fish breeders or culturists may want to breed males and females separately or to achieve a monosex culture depending on biological or economic traits. Hormonal induction of sex reversal may serve as a valuable tool to understand the process of sex differentiation and to produce monosex populations for the aquaculture industry.

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). Though such hormonal sex reversal has been achieved in as many as 47 species of fishes (Pandian and Sheela, 1995) , characids remain unventured for their artificial feminization with hormones , 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, to produce 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, as preliminary experiments with hormone administration through feeding (hormone mixed egg yolk or commercial finely powdered fish/prawn feed, 'FRIPACK') resulted in complete mortality of fry, even at the lowest level of 0.5mg/kg (data not given). This may possibly be due to the specificity for the type of feed in their very early stage of life or may be for some other unknown reason(s).

For treatment, a stock solution of hormone was prepared by dissolving the steroid (Sigma, USA)- Estradiol-17β(E-17β), a natural steroid mostly preferred for achieving feminization (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.

In short term immersion experiments, after exposure for a definite period, the fry were transferred back to rearing tanks. 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. 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). Females were identified by the presence of developing oocytes with lightly stained nucleus. 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.

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.

Feminization using Estradiol- 17β (E-17β)

Data on the effect of E-17β on the percentage of feminization are given in Table 1. Immersion treatments at doses ranging from 200-800 µg/l for 7 hours were found to be highly ineffective in producing 100% females. A dose of 600 µg/l however produced a significantly ($P<0.001$) increased percentage (61%) of females than the control (29%). A dose of 1000 µg/l proved lethal as all the fry succumbed within a week after exposure. The % survival rates of all the treated ones at doses 200, 400, 600, and 800 mg/l were almost similar to the control (Table 1); dose dependent mortality was not noticed.

With the increase in dose, there was decrease in % of intersexes and intersexes were never observed at the optimal dose of 600µg/l for 7 hours. Nevertheless intersexes reappeared at the dose of 800 µg/l.

With the other doses being lethal (200, 400, 800 µg/l), a dose of 600 µg/l for 10 hours significantly ($P<0.001$) increased the percentage of females (71%) than the control

Volume : 4 | Issue : 4 | Apr 2014 | ISSN - 2249-555X

(29%), however with a poor survival rate (11%) than the control (35%).

Identification of sex

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

DISCUSSION

Maximum feminization of 71% females has been obtained in the present study with 600 µg/l of Estradiol-17b for 10 hours of single short term immersion. In other studies, 68-100% females are secured with estradiol in fishes like S.trutta (Ashby, 1957), O.masou (Nakamura, 1981), O.kisutch (Piferrer et al., 1994) and Cyclopterus lumpus (Martin – Robinchand et al., 1994). Securing 100% females is found to be difficult in the present study, which may be due to low efficiency of the natural estrogen used. Strict dose dependent mortality has not been observed although the optimal dose (600mg/l for 10 hours) resulted in lower survival than the controls.

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 (Schreck, 1974; Nagy et al., 1981, Donaldson & Hunter, 1982; Varadaraj, 1990; Okako & Phelps, 1995). Appearance of intersexes may be species specific or in some cases may even be strain specific. While Nagy et al. (1981) have observed intersexes in the European strain of C.carpio, Ali and Rao (1989) observed no such intersexes in the Asian strain of C.carpio, with methyltestosterone treatment; may be that Asian strains are less vulnerable to hormone manipulation. On the basis of experiments with O.kisutch, Goetz et al. (1979) have suggested that the time of natural sex differentiation may vary considerably in different species and even between races.

ACKNOWLEDGEMENT

Author, B.Uma, would like to thank CSIR, New Delhi, for the financial support and Prof. M.R.Chandran, Bharathidasan University and Prof.T.J.Pandian , Madurai Kamaraj University , for the guidance and facilities provided.

Table- 1 : Effect of Estradiol - 17β (E-17β) administration through rearing water on the 5th day post hatchlings of G.ternetzi

S.No.	Dose (µg/l)	Initial No.	Survival % (at 75 days)	No. sexed	Sex distribution		
					F	I	M
I. Single short term immersion							
1.Duration : 7 hrs.							
(i)	200	100	37	17	0.29	0.12	0.59
(ii)	400	100	34	16	0.50	0.06	0.44 c
(iii)	600	100	36	13	0.61	-	0.39 a
(iv)	800	100	38	19	0.27	0.05	0.68
(v)	1000	100	-	-	-	-	-
2. Duration : 10 hrs.							
(i)	200	100	-	-	-	-	-
(ii)	400	100	-	-	-	-	-
(iii)	600	100	11	7	0.71	-	0.29 [a]
(iv)	800	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 C – P<0.05

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