RESEARCH PAPER | Zoology | Volume : 4 | Issue : 6 | June 2014 | ISSN - 2249-555X

Hormonal Sex Reversal in *Gymnocorymbus Ternetzi* (Boulenger) using Continuous Immersion of Estradiol -17β -- for Feminization

KEYWORDS: Hormonal sex reversal -Estradiol 17β - G.ternetzi

B.UMA

Department of Zoology, Bharathi Women's College, Chennai-600108, Tamil Nadu, India

ABSTRACT *Control population had a highly skewed sex-ratio in favour of males (59%), possibly due to temperature induced / based natural sex reversal.. With an increase in dose of continuous immersion of Estradiol-17β from 50-200μg/l (single exposure) there was corresponding increase in females from 17-40%. Intersexes were observed at all doses in addition to control stock. With double exposure (50-200μg/l), the percentage of females increased further significantly from 33 (P<0.005) to 44% (P<0.001). Survival rates were not much affected when compared to control except at a dose of 100 μg/l where it dropped to 17% and intersexes were not observed at any of the above doses. Sexes were identified through gonadal squash.*

INTRODUCTION

Improvement of methods for sex control ranks high in the priority list of aquaculturists, because there are differences in growth rate, behaviour pattern, breeding time, body colour, shape and size between male and female in each cultured species. Fish breeders or culturists may want to breed males and females separately or to achieve a monosex culture depending on biological or economic traits. 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). The direct strategy of producing monosex population through hormonal sex reversal has the advantage that the desired phenotypic adults are produced in the same generation as the treatment. In addition any gonadal sex may be produced (Hunter & Donaldson, 1983). However, hormonal sex reversal has so far been induced only in 47 species (Pandian & Sheela, 1995) and characids remain unmanipulated for artificial sex reversal using continous immersion of Estradiol -17β, hence the present study.

MATERIAL AND METHODS

Experimental fish

A vivacious, active, prized little 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. The growth was rapid and they became sexually mature at the age of 3.5-4 months.

Hormone administration

For treatment, a stock solution of hormone was prepared by dissolving the steroids (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 continuous immersion experiments, with single dose, required amount of stock solution was directly added into the rearing tanks (with known quantity of water) and the water was not changed at least for 4 to 6 weeks. In continuous immersion with double dose experiments, a second dose was added, one week after the first dose, to the same water and water was not changed here also for 4-6 weeks. 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).

STATISTICAL ANALYSES

Sex ratio differences were treated with Chi-square (χ^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.

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

Data on the effect of E-17β on the percentage of feminzation are given in Table 1. With an increase in dose from 50-200μg/l (single exposure) there was corresponding increase in females from 17-40% . Intersexes were observed at all doses. With double exposure (50-200μg/l), the percentage of females increased further significantly from 33 (P<0.005) to 44% (P<0.001). Survival rates were not much affected when compared to control except at a dose of 100 μg/l where it dropped to 17% and intersexes were not observed at any of the above doses.

Identification of sex

Sexes were identified based on the squash preparation of

Volume : 4 | Issue : 6 | June 2014 | ISSN - 2249-555X

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.

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 . The occurrence of various forms of Environmental Sex Determination (ESD) among fishes have been dealt in detail in a number of reviews (Shapiro, 1990; Francis, 1992). Thus , in the present case with G.ternetzi , 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, with an increase in dose of continuous immersion of Estradiol-17β from 50 to 200µg/l (single exposure) there was corresponding increase in females from 17-40%.. With double exposure (50-200µg/l), the percentage of females increased further significantly from 33 to 44% . Survival rates were not much affected when compared to control except at a dose of 100 µg/l where it dropped to 17% and intersexes were not observed at any of the above doses.

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 even with continuous immersion which may be due to low efficiency of the natural estrogen used. Strict dose dependent mortality has not been observed . 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).

ACKNOWLEDGEMENT

Author would like to thank CSIR (SRF) for the financial assistance , Professor M.R.Chandran , Bharathidasan University, Tiruchirappalli and Professor T.J.Pandian , Madurai Kamaraj University, Madurai , India for the guidance and lab facilities, provided.

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

S.No.	Dose (µg/l)	Initial No.F	Survival % (at 75 days) I	No. sexed M	Sex distribution		
1 Single dose:							
(i)	50	100	25	12	0.17	0.16	0.67
(ii)	100	100	27	13	0.39	0.07	0.54
(iii)	200	100	31	15	0.40	0.13	0.47
2. Double dose:							
(i)	50	100	30	15	0.33	-	0.67 b
(ii)	100	100	17	8	0.37	-	0.63 b
(iii)	200	100	36	18	0.44	-	0.56 a
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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