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Production of Hormone-induced Supermale of Genetically Improved Farmed Tilapia (GIFT-YY) in Bangladesh

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Abstract

A series of experiments were conducted to produce YY-supermale GIFT strain of tilapia in Bangladesh first, through optimization of feminizing hormone, Diethylstilbestrol (DES) to produce 100% female tilapia. The feminized female tilapia (XY) obtained from 150 mg/kg feed treatment were reared, tagged and bred individually. About 36% (4 out of 11 fish) of the treated fish became sex reversed female and it was confirmed by sexing their offspring, produced through crossing with genotypic male (XY), using aceto-carmine gonad squashing method that resulted in 75% male and 25% female. Since the 75% male offspring are supposed to contain 25% YY male, half of the offspring of each sex reversed females were feminized using DES at the same dose (150 mg/kg feed) to produce YY female by reversing the sex of the 25% YY male offspring. The stock that contained 75% male offspring was kept as it contained theoretically 25% YY male. The other half of the same stock (feminized half) that contained 25% YY female was also kept. Then the all of the individual male and female of such stocks were bred individually and identified the individual YY male and YY female by observing the sex ratio of their offspring as YY male or YY female that produced 100% genotypic male offspring in mating with genotypic female and male, respectively. A total of 106 fish from the non-feminized stocks were bred, 15 of them were identified as YY male but only 4 fish produced 100% male and the rest produced 80.12% to 97.18% male. Similarly, 45 fish from the feminized stocks were bred, 8 of them were identified as YY female but 3 of them produced 100% male and the rest produced 93.33% to 97.77% male. Finally the identified YY males and YY females were crossed to produce 100% YY supermales and thus the objectives were achieved.

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Introduction

In Bangladesh, commercial farming of tilapia has been found to develop rapidly since the introduction of Genetically Improved Farmed Tilapia (GIFT) from the Philippines in 1994 (Alam and Kawsar 1998). The success of using the GIFT strain of tilapia for commercial farming is due to its ability to produce millions of monosex male fry in hatcheries and this practice has been found to considerably eliminate the problems related to the production of mixed sex tilapia showing slow growth as well as the production of small-sized individuals in a given culture facility (Mair and Little 1991). The culture of mixed-sex tilapia can always give rise to unwanted reproduction that diverts the energy to gametic rather than somatic growth, resulting in a significant part of the harvest consisting of unmarketable juvenile fish, (Green et al. 1997), overcrowding and stunted growth (Pandian and Varadaraj 1990). However, by using the GIFT strain of tilapia, the culture of monosex males that show fast growth could be achieved. The technique of producing monosex males that is widely used in the reversal of sexually undifferentiated GIFT fry to genotypic males use 17-α methyltestosterone. Factors such as the concentration of hormone, treatment duration, age and size of fry as well as the availability of natural food, stocking density, feeding frequency, and age at first feeding can affect the production of 100% sex-reversed male fry (Mair and Little 1991).

The success in the production of 100% sex-reversed males using 17 α-methyltestosterone is also dependent on the method and frequency of feeding the hormone to the sexually-undifferentiated fry and controlling all the factors mentioned above that can affect the sex reversal process. Some concerns on environmental and human health due to the consumption of hormone-treated tilapia were raised (Mair et al. 1997) but no evidence for any human health hazard was found by consuming the hormone-treated tilapia (Green and Teichert-Coddington 2000). Exogenous steroids, such as the 17 α-methyltestosterone, are rapidly cleared from tissue after the end of treatment and no residual can be detected within one month of the termination of hormone treatment (Rothbard et al. 1990; Green and Teichert-Coddington 2000). The other method of obtaining single sex by manual separation involves intensive labour and high risk of human error in sexing, and hybridization between two tilapia species may not be able to produce hybrid vigor that shows fast growth (Mair and Little 1991).

The method used in the present study involves the use of genotypic YY male to cross breed with genotypic female (XX) for producing all genotypic male offspring. This method seems to be the most effective in obtaining monosex male if the genotypic YY-male and YY-female broodstock could be developed and maintained. Genetically male tilapia (offspring of YY male), herein known as the YY-supermale, has been reported to grow faster than the mixed-sex tilapia or the monosex tilapia obtained from the conventional hormone-induction method (Mair et al. 1997; Dr. Rafiqul Sarder, pers. comm.). The purpose of the present study is, therefore, to produce genotypically pure strains of YY-male and YY-female to be used as broodstock to produce all genotypic males (YY-supermales) for commercial culture.

Materials and Methods

Experiment site

The experiment was conducted for three years at the Research Station of Mennonite Central Committee (MCC) located in Noakhali, the eastern part of Bangladesh.

Experimental fish

The fish used in the experiment was produced in the MCC research centre but the parental stock was collected from Bangladesh Fisheries Research Institute (BFRI), Mymensingh.

Production of YY supermales

To produce YY-supermale tilapia a series of experiments were conducted. Figure 1 shows the protocol used in the production of YY supermales.

Series-I experiments

For optimizing the dose of Diethylstilbestrol (DES), GIFT fry were treated with different doses. Five diets (treatment) composed of fine powdered fish meal, with different doses of DES i.e., 0, 50, 100, 150 and 200 mg/kg were prepared through ethanol evaporation method (Mair and Santiago 1994) and 10 batches (each batch contained 200 hatchlings) of GIFT hatchlings were fed with the prepared diets after partial absorption of yolk sac. So each treatment had two replications. The fry was fed *ad libitum* for five times a day started at 8.30 am and continued up to 4.30 pm with two hours interval for 30 days. The fry was then transferred to *hapas* fixed in a pond and reared with the same feed without hormone until sexed at the age of 100 days. From each treatment group, representative samples were sexed using the aceto-carmine gonad squashing method as described by Guerrero and Shelton (1974) to observe the state of sex reversal. Treatments with 150 mg/kg and 200 mg/kg produced 100% female sex and these doses were considered as the optimum dose (Rahman and Sarder 2002). Twenty five females of the 150 mg/kg treatment group were reared up to maturity and used as brood females in the next step experiment. This hormone induced female brood stock contained both the sex reversed XY female and normal XX female.

Series-II experiments

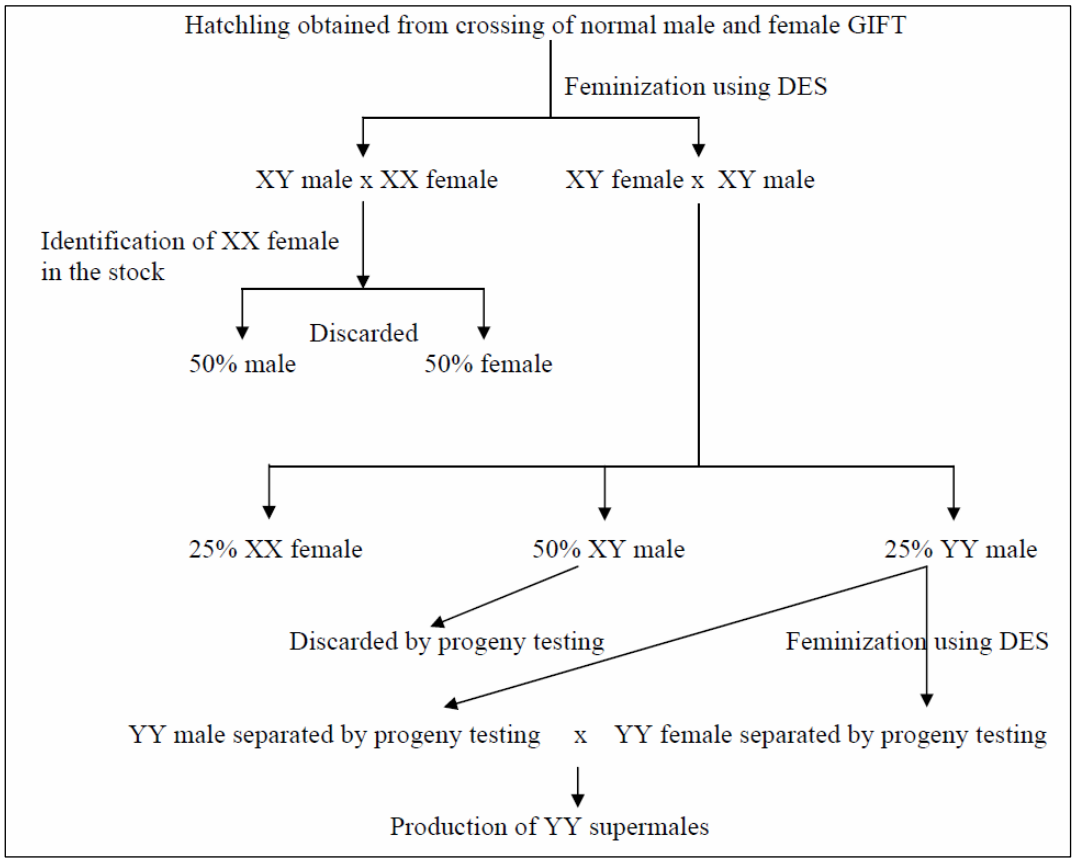
Since no genetic marker has been used the genotype of the DES induced 25 females from the series I experiment was determined by progeny testing. All the 25 females were kept individually in confinement with normal genetic (XY) males to facilitate the breeding. Out of 25 females, only 11 females were bred and produced offspring. These 11 females were denoted as

P-1 to P-11. The offspring of the females were sexed and four (36.36%) were identified as XY sex reversed females as they produced 75% male. These four sex reversed females were bred again and half of each of the offspring groups was feminized using DES @150 mg/kg feed, to produce sex reversed YY females. These stocks contained both XX and YY females and need to be separated. The other half of each offspring group was reared separately without any hormone and was supposed to be a mixture of XX female, XY and YY males. To identify the YY male, each individual male from these offspring groups was mated separately with normal female (XX) and the sex ratio of their offspring was checked. Males that produced 100% male sex were considered as YY males. They were tagged and reared.

Series-III experiments

To identify the YY females, all the females feminized with DES @150 mg/kg feed in series II experiment were bred individually in confinement with genotypic XY male and the sex of the offspring was checked. Females that produced 100% male sex were considered as YY females and tagged. Individual YY supermale was mated with individual YY female and a good number of YY supermales were produced.

Fig. 1. Schematic diagram of the protocol followed for the production of YY supermales.



Results and Discussion

A total of 25 DES treated females were reared to maturity and 11 of them were bred individually with normal males (XY). Out of eleven sex-reversed females, four were found to produce near to or 75% male offspring, based on chi-square test, indicating that the genotype of these sex-reversed females is to be XY (Table 1). The percentage of XY sex-reversed female was just 36.36% instead of the ideal 50% ratio because those females were randomly selected and many of them were kept in confinement for breeding. Length and weight of all the offspring groups were not in synchrony with their age because numbers of individuals in each stock were not the same but they were reared in the tanks of same size, so stocks with low density got more physical space and probably grew larger in size in shorter rearing period (Table 1). Though all the 25 females that were intended to be bred were phenotypically female, 14 out of these 25 females didn't respond to breeding and they were not subject to dissect their gonads. Therefore, it was difficult to comment whether they were completely reversed or had contained both the characters of ovary and testis in their gonad due to the DES treatment.

Only the offspring of 2 XY sex-reversed females were tested to find out individual YY male and YY female because these 2 XY sex-reversed females (numbered as P-6 & P-8 respectively, Table 1) have been found to produce the offspring, highly insignificantly different from the ideal 3:1 ratio in X^2 test.

Table 1. Identification of XY sex-reversed female produced near to or 75% male offspring.

Parent Female	offspring	O.N.	E.X.	€X^2 value	Tab. value	Result	Age at dissection	Wt. (g)	Length (cm)
P-1	Male	11	15	4.27	3.84 @ 5% level of	Sig.	125 days	13.6± 3.8	9.9 ± 0.6
	Female	9	5		Sig. df. 1				
P-2	Male	10	15	6.67	3.84 @ 5% level of	Sig.	123 days	16.5 ± 2.8	9.7 ± 0.5
	Female	10	5		Sig. df. 1				
P-3	**Male**	**32**	**37.5**	**3.23**	**3.84 @ 5% level of**	**Insig*.**	**116 days**	**6.2± 1.8**	**6.9 ± 1.2**
	Female	**18**	**12.5**		**Sig. df. 1**				
P-4	Male	6	15	21.6	3.84 @ 5% level of	Sig.	106 days	9.9 ± 0.2	8.3± 0.6
	Female	14	5		Sig. df. 1				
P-5	Male	11	15	4.27	3.84 @ 5% level of	Sig.	101 days	37.4 ± 4.8	14.3 ± 1.1
	Female	9	5		Sig. df. 1				
P-6	**Male**	**37**	**37.5**	**0.27**	**1.32 @ .1% level of**	**Highly**	**106 days**	**29.9± 5.1**	**11.9 ± 0.7**
	Female	**13**	**12.5**		**Sig. df. 1**	**insig*.**			
P-7	Male	16	22.5	6.71	3.84 @ 5% level of	Sig.	91 days	6.0± 1.2	7.1± 0.6
	Female	14	7.5		Sig. df. 1				
P-8	**Male**	**35**	**37.5**	**0.67**	**1.32 @ .1% level of**	**Highly**	**91 days**	**9.8± 1.7**	**8.4 ± 0.5**
	Female	**15**	**12.5**		**Sig. df. 1**	**insig*.**			
P-9	Male	11	15	4.27	3.84 @ 5% level of	Sig.	80 days	21.7 ± 5.9	10.4± 1.1
	Female	9	5		Sig. df. 1				
P-10	Male	6	15	21.6	3.84 @ 5% level of	Sig.	80 days	11.4 ± 3.4	9.0± 0.8
	Female	14	5		Sig. df. 1				
p-11	**Male**	**34**	**37.5**	**1.94**	**3.84 @ 5% level of**	**Insig*.**	**77 days**	**3.4 ±1.1**	**5.7 ± 0.6**
	Female	**16**	**12.5**		**Sig. df. 1**				

Sig. - Significant; Insig.*- Insignificant; O.N.-observed number; E.X.-expected number; €X^2- chi square value; Tab. value-Tabulated value

When 27 offspring of P-6 bred individually to identify YY male, five of them were identified as YY males, however, only three of them produced 100% male offspring, another two produced 96.42% and 82.48% male offspring, respectively, upon crossing with genotypic females (XX) (Table 2a). Similarly, a total of 79 offsprings of P-8 were bred individually and among them 10 were identified as YY males, however, only one of them produced 100% male offspring. Other nine males produced 80.21%, 97.6%, 87.75%, 86.44%, 96.87%, 87.27%, 97.18%, 96.97% and 88.88 % male offsprings, respectively (Table 2a). It has been reported that YY males of *Oreochromis niloticus* produced by androgenesis did not produce 100% male offspring when crossed with XX females (Myers et al. 1995; Sarder et al. 1999). Similarly, Ezaz et al. (2004) observed that androgenetically produced YY males did not produce 100% male offspring upon crossing with normal XX females and they assumed that other than the prime sex determining gene, some other sex modifying genes, e.g., autosomal genes and even different environmental factors could be responsible for changing the expected sex ratios.

Table 2a. Identification of YY supermale produced all-male offspring.

Parent: sex-reversed female (XY)	Male offspring of XY sex-reversed female which again used as YY male parent	Offspring sex ratio			% of male offspring
		Male	Female	Total	
P-6	N-3 H-15	27	1	28	96.42
	N-3 H-3	28	0	28	100.00
	P-6 M-3	146	31	177	82.48
	P-6 M-4	50	0	50	100.00
	N-3 H-19	48	0	48	100.00
P-8	N-2 H-8	73	18	91	80.21
	N-1 H-3	10	0	10	100.00
	N-1 H-40	83	2	85	97.60
	N-1 H-13	43	6	49	87.75
	N-2 H-38	51	8	59	86.44
	N-2 H-41	62	2	64	96.87
	N-2 H-45	48	7	55	87.27
	N-2 H-47	69	2	71	97.18
	N-2 H-49	64	2	66	96.97
	N-2 H-54	40	5	45	88.88

Out of 48 feminized offspring of P-6 that were intended to breed individually with normal genotypic males (XY), only 21 were bred successfully. Among these 21 feminized females, four were identified as YY females, of which two produced 100% male offspring and two produced 94% and 94.33% male offsprings respectively (Table 2b). Though all the 48 fish were phenotypically females, the 27 fish didn't breed within 8 months after maturity and were not dissected for sexing or confirmed as inter-sexed, but in similar cases 18.5% inter-sexed population has been reported (Karayucel et al. 2003).

Like P-6, the 58 feminized offsprings of P-8 that were intended to breed individually with normal gentotypic males (XY), 24 were bred successfully within six months when they attained maturity and four out of these 24 were also identified as YY females of which only one of them produced 100% male offsprings and the rest (3) produced 93.33%, 96.77% and 93.33% male offsprings, respectively (Table 2b). Similar to the previous case, those that didn't breed were not dissected for sexing and the reason of non-response to breeding was unknown.

Table 2b. Identification of YY female produced all-male offspring.

Parent: sex-reversed female (XY)	Feminized offspring of XY sex-reversed female which again used as YY female parent	Offspring sex ratio			% of male offspring
		Male	Female	Total	
P-6	N-3 H-44	85	5	90	94
	N-3 H-42	24	0	24	100.00
	N-3 H-27	92	0	92	100.00
	N-3 H-39	50	3	53	94.33
P-8	N-2 H-16	42	3	45	93.33
	N-2 H-12	60	2	62	96.77
	N-4 H-13	42	3	45	93.33
	N-4 H-23	60	0	60	100.00

The identified YY males and YY females were crossed and their offsprings were reared. A few of them were dissected and found as males (Table 3). As their parents were YY male and YY female, they were assumed to be all YY male, so they were raised to adult instead of sacrificing for sexing through dissection of gonad. The offspring of the parents N-3 H-19 x N-4 H-23 were crossed with genotypic female (XX) and produced 97.59% male offspring (data not shown).Thus the status of YY males produced from the crossing of YY males and YY females was assessed.

Table 3. Production of YY male offspring by crossing YY male and YY female parents.

No. of fish	No. of male offspring	No. of female offspring	Total fish dissected
P-6 M-4 * N-3 H-27	9	0	9
N-3 H-19* N-4 H-23	9	0	9
N-2 H-41* N-3 H-39	16	0	16
N-2 H-38* N-3 H-42	15	0	15
N-1H-40 * N-3 H-42	14	0	14

Conclusion

Culture of monosex male tilapia bears high production potential in Bangladesh. The monosex male tilapia fry could be produced either by hormonal sex reversal or by using YY supermale. A number of hatcheries are producing monosex male fry through androgenic hormone feeding, but a considerable percentage of female fry in each batch has been reported. Therefore it is important to produce YY supermales which will produce all-male progeny in one hand and on the other hand, the customers' concern on residual hormonal health hazard will be eliminated. The YY supermales can be produced through separating Y chromosome complements of hormone-induced XY females upon crossing with genotypic XY males in the two subsequent generations. The crossing of YY supermales with XX females will produce offsprings with only XY genotype. Thus the production of all male tilapias will be ensured.

Reference

Alam, S.M. and M.A. Kawsar. 1998. Effect of estrogens on growth and sex-ratio in the geneticallyimporved farmed tilapia, *Oreochromis niloticus* (L.); Bangladesh Journal of Zoology 26 (2): 37-43.

Ezaz, M.T., J.M. Myers, S.F. Powell, B. J. McAndrew and D. J. Penman. 2004. Sex ratios in the progeny of androgenetic and gynogenetic YY male Nile tilapia, *Oreochromis niloticus* L. Aquaculture 232:205-214.

Green, B.W., K.L. Veverica and M. Fitzpatric. 1997. Fry and Fingerling Production. In*:* Egna, H. and Boyd, C.E. (eds.)., Dynamics of Pond Aquaculture. Lewis Publishers. (peer reviewed)

Green, B.W. and D.R. Teichert-Coodington. 2000. Human food safety and environmental assessment of the use of 17 α-methyltesterone to produce male tilapia in the United States. Journal of World Aquaculture Society 31:337-357.

Guerrero III, R.D. and W.L. Shelton. 1974. An aceto-carmine squash method for sexing juvenile fishes. Progressive Fish Culturist 36 (1):56.

Karayucel, I., D. Penman, S. Karayucel and B. McAndrew. 2003. Thermal and hormonal feminization of all male YY nile tilapia, *Oreochromis niloticus* L. The Israeli Journal of Aquaculture- Bamidgeh 55(2), 114-122.

Mair G.C. and D.C. Little. 1991 Population control in farmed tilapia, Naga, ICLARM Quarterly, July 1991, pp 8-13.

Mair, G.C., L.R. Dahiling, E.J. Morales, J.A. Beardmore and D.O.F. Skibinski. 1997. Application of genetic techniques of the production of monosex male tilapia in Aquaculture: Early experiences from the Philippines. Proceedings of the Fourth Central America Symposium on Aquaculture, Tegucigalpa, Honduras, April 22-24, 1997, pp 225-227.

Mair, G.C. and L.P. Santiago. 1994. Feminization of Nile tilapia *Oreochromis niloticus* (L.) by oral application of Diethylstilbestrol (DES). In L.M. Chou, A.D. Munro, T.J. Lam, T.W. Chen, L.K.K. Cheong, J.K. Ding, K.K.Hooi, H.W. Khoo, V.P.E. Phang, K.F. Shim and C.H. Tan (eds.) The Third Asian Fisheries Forum, Asian Fisheries Society, Manila, Philippines: 94-97.

Myers, J.M., D.J. Penman, Y. Basavaraju, S.F. Powell, P. Baoprasertkul, K.J. Rana, N. Bromage and B.J. McAndrew. 1995. Induction of diploid androgenetic and mitotic gynogenetic Nile tilapia (*Oreochromis niloticus* L.). Theoretical and Applied Genetics 90 : 205-210.

Pandian, T.J. and K. Varadaraj. 1990. Development of monosex female *Oreochromis mossambicus* Broodstock by integrating gynogenetic technique with endocrine sex reversal. Journal of Experimental Zoology 255: 88-96.

Rahman M.M. and M.R.I. Sarder. 2002. Feminization of GIFT-first step to produce YY male in Bangladesh. Bangladesh Journal of Fisheries 25 (1-2): 193-198**.**

Rothbard, S., Y. Zohar, N. Zmora, B. Vevavi-Sivan, B. Moab, and Y. Yaron. 1990. Clearance of 17 α-methyltesterone from muscle of sex-inversed tilapia hybrids treated for growth enhancement with two doses of the androgen. Aquaculture 89:365-376.

Sarder, M.R.I., D.J. Penman, J.M. Myers and B.J. McAndrew. 1999. Production and propagation of fully inbred clonal lines in the Nile tilapia (*Oreochromis niloticus* L.). Journal of Experimental Zoology 284 : 675-685.

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