

## Breeding biology and monosex male seed production of Genetically Improved Farmed Tilapia (GIFT) strain of *Oreochromis niloticus* L. in Bangladesh

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### Abstract

A study on the breeding biology of the GIFT strain of Nile tilapia, *Oreochromis niloticus*, was conducted for a period of five months. The sex ratio of the parent individuals was optimized for performance in spawn production, where the best results were obtained with a sex ratio of female to male of 4:1 compared to those of 3:1 and 2:1. The diameter of eggs obtained from the GIFT stock had major and minor axes of  $2.19 \pm 0.09$  and  $1.72 \pm 0.07$  mm, respectively, with no significant differences between the treatments. The average number of eggs produced was  $392 \pm 22$  per female, with fertilization and hatching rates ranging between 94-96% and 85-88%, respectively. No significant variation was observed between the treatments. Breeding frequencies per female in the three treatment groups ranged between 10-40 days and the highest value was obtained at a female to male sex ratio of 4:1. In an other experiment, 17  $\alpha$ -Methyltestosterone (MT) was applied orally to the fry at their first feeding stage with treatments- 1, 2, 3 and 4 at the dosage of 100, 80, 60 and 40 mg/kg feed respectively, for the period of 28 days. The mean percentage of males obtained in treatments, 1, 2, 3 and 4 were 98, 97, 95 and 68, respectively. Treatments-1, 2 and 3 did not differ significantly ( $P > 0.05$ ) from each other but treatment 4 showed significant variation ( $P < 0.05$ ) from other treatments. The results showed that MT-100, 80 and 60 mg/kg feed administered for 28 days produced close to cent percent male population of the GIFT strains in aquaria.

**Key words :** Breeding biology, GIFT, Monosex tilapia

### Introduction

The name 'Tilapia' was derived from an African word simply meaning 'fish'. These fishes belong to the family 'Cichlidae' (Steeba 1962). There are about 100 species and most of them are native to Western African rivers (Anon 1984). Only 16-23 species are suitable for culture in ponds (Huet 1970, Balarin and Hatton 1979). Of these, only *Tilapia* (*T. zillii* and *T. rendali*) and *Oreochromis* (*O. niloticus*, *O. mossambicus* and *O. aureus*) species are in widespread use (Hepher and Pruginin 1982). Among the wide

variety of cultured tilapias, the most widely farmed species is the Nile tilapia (*O. niloticus*).

Tilapias are recognized as one of the most important fish species for farming in a wide range of aquaculture systems from single small scale waste fed fish ponds to intensive culture systems (Pullin 1985). Tilapias have been dubbed the 'aquatic chicken' (Maclean 1984). They form the mainstay for many poor fish farmers.

Recently, the WorldFish Center (formerly ICLARM), Philippines developed the Genetically Improved Farmed Tilapia, known as the GIFT strain through several generations of genetic selection from a base stock involving eight different *O. niloticus* strains. The strain was introduced into Bangladesh in 1994 for genetic evaluation and further selection.

As the tilapia is a promising culture species and the GIFT is an introduced improved strain, the fish is very much important for evaluation regarding their performances as well as some important aspects of their breeding biology. No attempt was made to assess the impact of any of the environmental and social parameters or breeding behaviour of the GIFT fish in Bangladesh. Bangladesh Fisheries Research Institute (BFRI) at its Freshwater Station (FS), Mymensingh, therefore, initiated research on evaluation of the GIFT strain on their growth and culture performance (Hussain *et al.* 2000).

The major constraint in raising tilapias is their uncontrolled reproduction in grow-out ponds that leads to overcrowding, competition for available food and stunting in growth in aquaculture systems, thus making this valuable species commercially useless in some situations. The use of monosex culture is the management practice most widely used to prevent this happening. In tilapias, the males grow faster than the females (Guerrero and Guerrero 1975) and the superior growth of male tilapia seems to be genetically controlled. Therefore, monosex culture eliminates the danger of uncontrolled reproduction and also gives higher yields (Hussain 2004). Many approaches for controlling reproduction have been tried and the most successful method is hormonal induction of monosex populations (Hunter and Donaldson 1983).

The present studies therefore attempted to evaluate some attributes in breeding biology especially in relation to optimization of sex ratio of brood individuals in a suitable controlled breeding environment for spawning and thereafter, testing of male hormone in sex reversal of the GIFT strain of Nile tilapia.

## Materials and methods

### *Breeding biology*

The study was conducted at the Freshwater Station (FS) of the BFRI, Mymensingh for a period of five months during May to September'00.

### *Origin of the fish stock*

The GIFT strain was developed by the International Center for Living Aquatic Resources Management (ICLARM) through several generations of genetic selection

from a base population involving eight different strains of Nile tilapia, *O. niloticus* (Eknath *et al.* 1993). In on station trials, the synthetic GIFT strain was reported to show on an average 60% faster growth and 50% better survival at harvest than the most commonly farmed strains in the Philippines, the Israel strain (Eknath 1992). For evaluating this strain in other countries of Asia, a research project was initiated in five INGA member countries *viz.* Bangladesh, China, Philippines, Thailand and Vietnam under the auspices of a WorldFish center's (Formerly ICLARM) project entitled 'Dissemination and Evaluation of Genetically Improved Tilapia Species in Asia (DEGITA)'. As a member of INGA, BFRI received GIFT strain during July'94.

**Stocking and management of breeders :** The experiment was conducted in nine nylon hapas (4.0 m<sup>3</sup> each submerged in water; 3.4 m x 2.0 m x 0.6 m) over a period of five months. There were three treatments on sex ratio of broods (Female: Male) with three replicates T<sub>1</sub> (2:1), T<sub>2</sub> (3:1) and T<sub>3</sub> (4:1). All brood fish were PIT (Passive Integrated Transponder) tagged before initiation of the trial. All the hapas were placed in a pond (1600m<sup>2</sup>) and brood fish (80-90g) were stocked in the hapas at a density of 3 fish/m<sup>3</sup>.

All the fish were fed daily with a formulated feed (40% rice bran, 15% mustard oil cake, 30% wheat flour and 15% fish meal) at the rate of 6% of estimated body weight. The quantity of feed given to fish was adjusted every two weeks. Water quality parameters such as temperature (°C), dissolved oxygen (mg/l) and pH of hapa water were measured between 8.00 to 9 00 AM at weekly intervals during the experimental period.

**Experimental procedure :** The mouths of females in hapa were checked at ten days interval to observe their breeding performance. Eggs were collected from the mouth of a female and the tag number of the spent fish was recorded. The total number of eggs collected from a female was counted and the rate of fertilization of eggs was estimated through observations under stereo microscope, the dimensions of eggs (both major and minor axis) was determined with an eye piece micrometer (Olympus Compound Microscope; Olympus CHT, Olympus Optical Co., Ltd., Japan).

#### *Monosex seed production of GIFT strain*

**Egg collection and spawn production :** Fertilized eggs collected from the mouth of a female from the breeding hapas were kept in jar (6 L) incubation system in hatchery with a water flow rate of 2 L per minute. Fry were obtained through hatching of eggs after an incubation period of 90-96 hours. The hatchlings were transferred through water flow of the jar to a metal trough with showering adjacent to the hatching jar.

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**Hormone treatment :** Six day old fry were divided into fifteen groups for five treatments with three replicates each containing 200 individuals in glass aquaria (50x23x23 cm<sup>3</sup>). The hormone 17 $\alpha$ -Methyltestosterone was administered orally to the fry at their first feeding stage to treatments 1, 2, 3 and 4 at the dose of 100, 80, 60 and 40 mg/kg feed. Feeding was *ad libitum* for the first 14 days and at 10% body weight for the following 14 days. The fry of treatment 5 (control) were fed with the hormone free diet. Water in each aquaria was partially changed (30%) once daily in the morning during the removal of uneaten food and faeces. The hormone feeding was continued for a period of 28 days.

**Fry nursing in hapa :** After hormone treatment, the different groups of treated and non-treated fry were transferred to hapas placed in a pond for further rearing with hormone free diet. During this nursing period, all the treated groups were given supplementary feed containing 30% protein at a feeding rate of 8%. After 8 weeks of rearing, all surviving fry were harvested and sexed through gonadal examination.

**Gonad examination :** Samples of 30% of the individuals of each replicate of the treated and non-treated fish were dissected to take out the gonads to examine under a compound microscope. Acetocarmine stains was used on the slide for clear identification of the gonad.

*Statistical analysis*

Data were analysed using the statistical package, Statgraphics Version 7. Duncan's New Multiple Range Test (DMRT) was applied to identify which treatment was different from others. Goodness-of-fit  $\chi^2$  tests were used to assess the fit of observed numbers of males and females to a 1:1 sex ratio.

**Results**

*Water quality parameters*

Mean values for temperature (°C), dissolved oxygen (DO), and pH of hapa water during the experimental period were observed to be 29.50 $\pm$ 1.87 to 31.20 $\pm$ 1.26°C, 4.84 $\pm$ 0.77 to 5.74 $\pm$ 0.67 mg/L and 7.05 to 8.31, respectively. Table 1 represents the records for the above characteristics of physicochemical parameters.

**Table 1.** Egg diameter, average fertilization and hatching rate of GIFT

| Treatments     | Sex ratio of brood individual |      | Egg diameter ( $\mu$ m) |                 | Average fertilization (%)  | Average hatching (%)       |
|----------------|-------------------------------|------|-------------------------|-----------------|----------------------------|----------------------------|
|                | Female                        | Male | Major axis              | Minor axis      |                            |                            |
| T <sub>1</sub> | 2                             | 1    | 2.20 $\pm$ 0.90         | 1.70 $\pm$ 0.08 | 96 $\pm$ 4.21 <sup>a</sup> | 87 $\pm$ 5.31 <sup>a</sup> |
| T <sub>2</sub> | 3                             | 1    | 2.17 $\pm$ 0.89         | 1.72 $\pm$ 0.09 | 94 $\pm$ 4.43 <sup>a</sup> | 85 $\pm$ 6.88 <sup>a</sup> |
| T <sub>3</sub> | 4                             | 1    | 2.19 $\pm$ 0.87         | 1.70 $\pm$ 0.08 | 93 $\pm$ 2.96 <sup>a</sup> | 84 $\pm$ 7.33 <sup>a</sup> |

\*Figures in a same column with a same superscripts are not statistically different (p>0.05)

*Egg diameter, fertilization and hatching*

The mean diameter of egg (major and minor axis), average rates of fertilization and hatching are shown in Table 2. No significant differences ( $p > 0.05$ ) were found in the axes of egg among the three treatments in which the ranges obtained as major and minor axis were found to be 2.17-2.20 and 1.70-1.72, respectively. The values of the rate of fertilization and hatching did not show any significant variations ( $p > 0.05$ ) among the treatments.

Table 2. Total number of egg and spawn produced per female of GIFT

| Treatments     | Sex ratio of brood individual |      | Total no. of eggs produced per female | Total number of spawn produced per female |
|----------------|-------------------------------|------|---------------------------------------|---|
|                | Female                        | Male |                                       |   |
| T <sub>1</sub> | 2                             | 1    | 369±83 <sup>a</sup>                   | 317±42 <sup>a</sup>                       |
| T <sub>2</sub> | 3                             | 1    | 412±92 <sup>a</sup>                   | 346±55 <sup>a</sup>                       |
| T <sub>3</sub> | 4                             | 1    | 396±68 <sup>a</sup>                   | 337±38 <sup>a</sup>                       |

\*Figures in a same column with a same superscripts are not statistically different ( $p > 0.05$ )

*Fecundity and spawn production*

The average rates of production of eggs in a single spawning by a female and thereafter production of spawn among treatments are shown in Table 3. The range of egg production in all the treatments was found to be more or less similar and also the mean values showed no significant variation. In case of spawn production, all the treatments show similar results.

Table 3. Average percentage of female individual bred, average breeding frequency of a female and range of breeding interval of GIFT

| Treatments     | Sex ratio of brood individuals |      | Female individual bred (%) | Breeding frequency of female | Range of breeding intervals (day) |
|----------------|--------------------------------|------|----------------------------|------------------------------|-----------------------------------|
|                | Female                         | Male |                            |                              |                                   |
| T <sub>1</sub> | 2                              | 1    | 63.0±4.58 <sup>b</sup>     | 1.83±0.28 <sup>c</sup>       | 10-40                             |
| T <sub>2</sub> | 3                              | 1    | 72.33±13.32 <sup>b</sup>   | 2.53±0.21 <sup>b</sup>       | 10-40                             |
| T <sub>3</sub> | 4                              | 1    | 94.33±5.91 <sup>a</sup>    | 3.50±0.86 <sup>a</sup>       | 10-40                             |

\*Figures in a same column with a same superscripts are not statistically different ( $p > 0.05$ )

*Breeding frequency and interval*

Among the three treatments, the average percentage of spent females and breeding frequencies along with observed ranges of breeding intervals of them within the period are shown in Table 4. The breeding performances and breeding frequencies were observed over a period of 150 days. In the females, 63, 72 and 94% individuals were bred in T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub>, respectively. In case of breeding frequency, T<sub>3</sub> showed the highest value

of 3.50 while, T<sub>1</sub> and T<sub>2</sub> were 1.83 and 2.53, respectively. These results showed significant differences ( $p < 0.05$ ) among the three treatments. The data of breeding intervals in all the treatments were observed to be in a same range of 10-40 days.

**Table 4.** Effects of different levels of 17  $\alpha$  Methyltestosterone (17 MT) administration on sex ratio of GIFT strain

| Treatments                  | No of fry stocked | End of MT treatment            |                 | Post treatment reared in hapa |                 |                 | Sex (%) |  |
|-----------------------------|-------------------|--------------------------------|-----------------|-------------------------------|-----------------|-----------------|---------|--|
|                             |                   | Mean wt. (mg)                  | Survival (%)    | Mean wt. (g)                  | Survival (%)    | Male            | Female  |  |
| T <sub>1</sub>              | 200               | 24.46 $\pm$ 13.04 <sup>a</sup> | 90 <sup>a</sup> | 2.97 $\pm$ 1.39 <sup>a</sup>  | 88 <sup>a</sup> | 98 <sup>a</sup> | 02      |  |
| T <sub>2</sub>              | 200               | 25.34 $\pm$ 5.92 <sup>a</sup>  | 92 <sup>a</sup> | 2.77 $\pm$ 0.90 <sup>a</sup>  | 86 <sup>a</sup> | 97 <sup>a</sup> | 03      |  |
| T <sub>3</sub>              | 200               | 26.18 $\pm$ 7.19 <sup>a</sup>  | 92 <sup>a</sup> | 3.13 $\pm$ 0.87 <sup>a</sup>  | 89 <sup>a</sup> | 95 <sup>a</sup> | 05      |  |
| T <sub>4</sub>              | 200               | 23.89 $\pm$ 8.22 <sup>a</sup>  | 90 <sup>a</sup> | 3.04 $\pm$ 1.52 <sup>a</sup>  | 90 <sup>a</sup> | 68 <sup>b</sup> | 32      |  |
| T <sub>5</sub><br>(control) | 200               | 25.98 $\pm$ 16.07 <sup>a</sup> | 92 <sup>a</sup> | 3.24 $\pm$ 0.78 <sup>a</sup>  | 89 <sup>a</sup> | 34 <sup>c</sup> | 66      |  |

\*Figures in a same column with a same superscripts are not statistically different ( $p > 0.05$ )

### *Sex reversal of GIFT strain*

Data on hormone treatment, mean weight, survival and sex ratio are presented in Table 5. The mean weight of fry of treatments 1, 2, 3, 4 and 5 at the end of the 28 day hormone feeding phase were 24.46 $\pm$ 13.04, 25.34 $\pm$ 5.92, 26.18 $\pm$ 7.19, 23.89 $\pm$ 8.22 and 25.98 $\pm$ 16.07 mg, respectively. No significant differences in mean weight were observed between control and treated groups, indicating that hormone had no effect on the early growth phase of GIFT fry. The survival rates of all groups of fry were not significantly different, which suggests that the hormone did not affect survival of fry. In the nursing phase, the survival rate was also not significantly different between the groups.

Each hormone treated group gave a mean male: female that deviated significantly ( $\chi^2$   $p < 0.05$ ) from the normal 1:1 ratio, with the male significantly higher than the female. The control group showed an unexpectedly high proportion of female (i.e.66%). The mean percentage of males of treatments, 1, 2, 3, 4 and 5 were 98, 97, 95, 68 and 34, respectively. Treatments-1, 2 and 3 did not differ significantly ( $p > 0.05$ ) from each other but treatment 4 showed significant variation ( $p < 0.05$ ) from treatments-1, 2 and 3. All the MT treated groups were found to be significantly different from the control groups. The results demonstrate that the dose of MT-100, 80 and 60 mg/kg feed for 28 days is sufficient to produce close to 100 percent male population of the GIFT strains in aquaria.

## Discussion

The present experiment has given some idea on breeding biology especially in some reproductive traits through varying the sex ratio of brood individuals in a suitable controlled breeding environment for spawning. Additionally, the present study also included experimentation on the production of monosex population to reduce the problems of overcrowding in culture ponds through sex reversion from female to male.

In published studies, the sex ratio of brood fish (Female: Male) were at 10:1 in hapas and tanks, whereas the ratio was 5:1 in ponds. For synchronized spawning, fewer than five females for every male appeared to be within the optimal range and less than three females appeared also optimal (Little and Hulata 2000). Frequency of spawning of tilapia in their natural environment depends on environmental variables (Rana 1988, Macintosh and Little 1995). Female tilapias are capable of spawning every 4-6 weeks under ideal condition in their natural environment (Jalabert and Zohar 1982, Little *et. al.* 1993).

The present experiment showed dissimilar results in the breeding frequencies and also in breeding intervals among the three treatments. The female: male sex ratio of 4:1 ( $T_1$ ) produced higher breeding frequencies and lower intervals compared to 3:1 and 2:1 ( $T_2$  and  $T_3$ ). These results are consistent with those of Little and Hulata (2000), Jalabert and Zohar (1982) and Little *et. al.* (1993).

The sex ratio of brood fish in  $T_1$  indicated that the competition for courtship was cooperatively higher. A lower value of breeding frequency as well as a higher value of breeding interval, therefore, could be obtained in the group and the situation make possibilities of a decreased rate of spawn production. Likewise, as the competition for courtship was comparatively lower in  $T_3$  under which ripe males were most continuously available waiting for a new female to encounter courtship and breeding. Thus, there is a chance of occurring higher performance/frequency of breeding as well as a lower value of breeding interval in the group and the situation makes possibilities of an increased rate of spawn production.

The number of egg produce by a female at a time can be varied with the strain, age and weight and, also the environmental factors. The fertilization rates were estimated to be the live eggs as counted among total number of eggs collected from a female's mouth, however, the results showed significant variations among the treatments; but significant differences were found in the rates of hatching and spawn production.

The size of egg can be a variable normally depending on age, weight and strain of the parents and the present results were found to be more or less same because of the fish under the three treatments were of same strain, ages and similar sizes. The size frequency distribution of the eggs obtained from the three treatments showed the modal classes to fall into the same class interval as being the major axis, 2.0-2.2 mm and minor axis, 1.5-1.7 mm.

The effectiveness of sex reversal treatment depends on the species, age at which hormone is administered, type and dosage of hormone, time and duration of treatment (Ridha and Lone 1990). Jalabert *et. al.* (1974) reported that 100% of male sex was achieved with a hormone dose at 40 mg MT/kg feed. A concentration of 30 mg MT/Kg

diet has been reported to achieve complete sex reversal in *O. mossambicus* (Guerrero 1979). Oral administration of MT-treated feed (30 to 60 mg MT/kg feed) to tilapia fry during a 3 to 4 weeks period yields populations composed of >95% males (Shelton *et al.* 1978, Tayamen and Shelton 1978, Guerrero and Guerrero 1988, Green and Lopez 1990, Phelps *et al.* 1992, Hiott and Phelps 1993 and Hussain 2004)). Moreover, incorporation of 90, 100 or 120 mg MT/kg feed did not enhance, accelerate or depress sex inversion of tilapia fry when compared to results obtained with 30-60 mg MT/kg feed (Nakamura and Iwahashi 1982, McGeachin *et. al.* 1987). The results of the present findings are consistent with findings of the authors that mentioned above.

A study with oral administration of 30-60 mg MT/kg feed to tilapia fry did not induce toxic effects and also growth and survival of tilapia fry fed on treated diet (60 mg MT/kg feed) for sex inversion were similar to fry fed androgen free diet (Green and Teichert-Coddington 1994). In another experiment, McGeachin *et. al.* (1987) mentioned that Tilapia fry offered a diet that contains 0, 60, 90 or 120 mg MT/kg feed for 22-d had similar survival rates. In an earlier study, Guerrero (1975) did not observe significant difference in survival between control fry and MT treated (0, 15, 30 or 60 mg MT/kg feed) fry after a 21-d treatment period. In fact, no significant difference in growth or survival were noted between MT treated and untreated fish during the experimental period of the present study. Further research needs to be carried out to optimize the duration of hormone administration while keeping the present dose (60-100 mg MT/kg feed).

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A.H.M. Kohinoor *et al.*

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