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Upgradation of genetically improved farmed tilapia (GIFT) strain by family selection in Bangladesh

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Abstract
The aim of the present study was to evaluate growth performance of the Genetically Improved Farmed Tilapia (GIFT) strain after six generations of genetic selection for increasing body weight. The founder stock comprised of 30 families having 300 individuals of the GIFT strain from Malaysia in 2005. The brooders (40 females and 40 males) with the highest breeding values in the founder stock were selected to produce progeny of the first generation (G_1) in 2007. From each family 25 each male and female fingerling was randomly Passive Integrated Transponder (PIT) tagged and reared in a pond for continuation of the selection program. The same protocol was followed in subsequent generations in 2008, 2009, 2010, 2011 and 2012. General linear model analysis indicated that the selected fish had 7.17, 13.60, 23.21, 30.30, 35.38 and 39.25% greater harvest weight than that of the founder population in G_1, G_2, G_3, G_4, G_5, and G_6 generations, respectively. This achievement greatly contributed to sustainable increase of tilapia production in Bangladesh.

Keywords: Stock Improvement, Family Selection, GIFT strain, Growth Performance Evaluation

1. Introduction
Tilapia has become the shining star of aquaculture with farms starting and expanding across the globe while consumption races ahead of even the most ambitious farm building plans. Farmed tilapia productions in 2010 exceeded 3.2 million metric tons per annum, surging further ahead of the salmon and catfish industries [1]. Production of tilapia, for home or local consumption and for export, has risen tremendously in the last few decades. The tonnage of world wide tilapia production (in 2010, about 3 million tons) is second, among fish, only behind to carps. Global production of tilapia was estimated to be 2.5 billion US$ in 2010 [2]. In view of the increasing commercialization and continuing growth of the tilapia industry, the commodity is not only the second most important farmed fish globally, next to carps, but is also described as the most important aquaculture species of the 21st century [3]. The fish is being farmed in about 85 countries worldwide, and about 98% of tilapia produced in these countries is grown outside their original habitats [4]. In INFOFISH Tilapia 2010 Conference it was forecasted that the world's total tilapia production would reach 3.70 million tones by the end 2010. The main culture industries are in the Far East but tilapias are increasingly being farmed in the Caribbean, Latin America and recently, in temperate countries where warm water through artificial means (thermal effluents or geothermal springs) are also available.
The development of Genetically Improved Farmed Tilapia (GIFT) technology that is based on traditional selective breeding as a means to improve commercially important traits of tropical farmed fish is a major milestone in the history of tilapia aquaculture [5]. The GIFT was developed by ICLARM (currently WorldFish) through several generations of selection from a base population involving eight different strains of Nile tilapia [6, 7]. Bangladesh Fisheries Research Institute (BFRI) received a sample of the GIFT strain in 1994 and further 16 families in 1996 through WorldFish Center (formerly ICLARM). In on-station and on-farm trials of BFRI, the GIFT strain was reported to show 35-57% superior growth than that of the existing strain in the country [8]. Further stock improvement of GIFT through mass selection was initiated in 1998. Six generations (G_1 to G_6) were produced through mass selection. Through combined selection technology, the G_6 generation of GIFT strain achieved 3.7% higher growth over the existing GIFT strain. The rate of genetic gain in weight of fish was greater up to G_3 but gradually decreased after that up to the sixth generation.

The reason behind such a decrease in genetic gain, particularly for body weight, might have been the accumulation of inbreeding. Therefore, the genetic improvement strategy for GIFT at BFRI was re-designed in 2005. Now the stock improvement program is being implemented through a family selection protocol under the technical assistance of WorldFish Center. In this paper we highlight the results of growth of the improved GIFT strain in different generations through the family selection protocol.

2. Materials and Methods

Stock improvement through family selection

2.1 Origin of stock

A new founder stock comprising of 30 families having a total of 300 individuals of new generation of GIFT strain have been further introduced from Malaysia through WorldFish Center in March 2005. The founder stock fish were reared in a 100m^2 hapas for three months. After three months rearing, the mean weight of female and male were 41.18±5.41 and 30.42±3.47 g, respectively.

2.2 Tagging of founder stock

Passive Integrated Transponder (PIT) were used for individually tagging the females and males. A PIT tag was injected into the peritoneal cavity of a fish and the number of the tag was recorded. After tagging all the fish were transferred to a 1000 m^2 pond.

2.3 Communal rearing in pond

After tagging, the male and female fish were fed with supplementary feed (28% crude protein) six days a week, at a rate of 4-5% of estimated biomass. Fish sampling was done at fortnightly intervals to assess the growth and for feed ration adjustment. Water was supplied once a week to maintain water depth at 1.0 meter. The pond was fertilized fortnightly with Urea and Triple Super Phosphate at the rate of 12.5 and 25.0 kg/ha, respectively. After four months of rearing, the fish were recaptured through seine netting and pond drying. The harvest body weight, sex and tag number of all harvested fish were recorded.

2.4 Estimation of breeding value

Breeding values were estimated for individual fish in a full pedigree, using SAS (SAS Inc, 1997) and ASREML [9]. The statistical model included the fixed effect of sex because this effect on size and growth is often found in aquaculture species [10]. Random term in the model was the additive genetic effect of individual fish in the pedigree. Age from birth to harvest was fitted as a linear covariate.

2.5 Breeding in hapas for G_1 (Generation 1) production

After harvest, genetic evaluation using the statistical model as described above was carried out on 300 founder animals to estimate their breeding values (EBVs). On the basis of breeding values of the founder stock, the best 40 males and best 40 females (from 30 families) were mated for the production of G_1 generation. For breeding, 40 breeding hapas (1.0m^3) were set up in a pond with bamboo poles. A pair of female and male breeders (1:1) was stocked in each breeding hapa. After 12 days from stocking, fertilized eggs were collected from the mouths of brooding females. Collected batches of eggs were transferred to the hatchery for separate incubation. Immediately after hatching, the larvae were transferred to a series of trays (one for each family) and were kept until their yolk sac resorption stage.

2.6 Nursing in hapa

From each family, 300 fry were transferred to an individual fine-mesh nursery hapa (2 m^3) in a pond. The progeny were fed with nursery feed containing 30% protein at the rate of 30% of estimated body weight. The mean weight of the fry was 2.80±0.42 g after 45 days of nursing.

2.7 Rearing in hapa

Subsequently, 150 fry from each progeny group were transferred to an individual rearing hapa (2.0 m^3 in size). Supplementary feed (Nursery feed) was applied in all the hapas at the rate of 15% of estimated biomass. After sixty days of rearing the weight range for male and female were 36-43 and 28-32 g, respectively.

2.8 Experimental Procedures

Each progeny group 25 male and 25 female fish were randomly sampled and tagged using Passive Integrated Transponder (PIT). Tagged fish from 40 families (2000 fishes) were stocked in a pond having 1000 m^2 area for communal rearing. Supplementary feed (25% crude protein) was supplied regularly at the rate of 6% of estimated biomass. The fish were harvested and tag number, weight, sex, body depth were recorded after six months of grow-out in pond. Then, breeding values of G_1 generation were estimated from the complete data set, tracing back to the foundation population (G_0).

2.9 Evaluation of gain in growth performance in G_1 generation of GIFT strain

The growth performance of progeny of the selected (G_1) fish and progeny of the non-selected population (founder stock; G_0) were compared in concrete cisterns (2.0 m^3) for a period of four months during April to July 2007. After tagging, surplus selected fish were sampled for this experiment. By contrast, the non-selected population (200 breeders) was stocked in a 300 m^2 pond for mass breeding. After 40 days from stocking, 6,000 fry were collected and reared in a 10 m^3 hapas for a period of three weeks. From this population, fry samples were taken for growth comparison. The initial mean weights of the selected fish and of the founder population (non-selected population) was 2.95±0.65 and 2.65±0.82 g, respectively. There were two treatments with three replicates. Before stocking the cisterns were cleaned and filled up with deep tube well water at the depth of 1 m. Fingerlings were stocked at a density of five fish/m^3.

Fish were fed twice a day, six days a week, with supplementary feed (28% crude protein) at 5-8% body weight. During grow out period, first and second months, feed was given at the rate of 8% and 7% of body weight, respectively, then subsequently, 6% and 5% feed were given to the fish in the 3rd and 4th month, respectively. Fish sampling was done at monthly intervals to assess the growth, and feeding ration was adjusted on the basis of estimated weight of fish biomass. Average water depth was maintained in all the cisterns at 1.0 m during the experimental period. After five months rearing, all the fishes were harvested. After harvest, body weight was measured on individual fish. Statistical analysis was carried out to test significant differences in growth between the G_1 generation fish and the founder stock (G_0).

2.10 Production of G_2 (Generation 2) generation of GIFT strain

On the basis of breeding values of G_1 generation, the best 60 males and 60 females were mated. For breeding, 60 breeding hapas (1x1x1 m) were set up in a pond. The range of breeding

values for body weight of selected males were 8.23 to 16.13g, while in case of females, the values were 5.01 to 14.47g. A pair of female and male breeders (1:1) was stocked in each breeding hapa. After 21 days of stocking, 300 larvae from each progeny group were transferred to a series of hapas in a pond. The progeny were fed with nursery feed containing 30% protein at the rate of 30% of estimated body weight. After 30 days nursing, the mean weight of the fry was 2.46±0.81 g. Each progeny group, 150 fry were transferred to 70 individual 2 m^3 size rearing hapas. Supplementary feed (Nursery feed) was applied in all the hapas at the rate of 10-15% of estimated biomass. After 1.5 months of rearing, the weight ranges of fingerlings were 15-22 g.

2.11 Tagging and communal rearing

Twenty male and 20 female sampled from each progeny group were tagged by using Passive Integrated Transponder (PIT). Tagged fishes from 60 families (2400 fishes) were stocked in a pond (1000 m^2) for communal rearing in 1 July 2008. The tagged fishes were reared in the pond. Supplementary feed (25% crude protein) was supplied regularly at the rate of 6-8% of estimated biomass. After four months rearing, fish were harvested and tag number, weight, sex, body depth were recorded. Then data were analyzed through statistical analysis for breeding value estimation. Mating of closely related individuals was avoided to minimize inbreeding. The same production and selection procedures were followed in subsequent generations G_3, G_4, G_5 and G_6. From G4 generation, evolutionary algorithm (EVA) software [11] was used to manage inbreeding in the selected population.

2.12 Evaluation of growth performances between upgraded GIFT strain (G_2) and founder population in pond

For evaluating the growth performances of upgraded GIFT strain (G_2) and founder population of GIFT strain in a pond for a period of five months during June to November 2008. A pond having an area of 1000 m^2 was selected for growth performance evaluation. Prior to the evaluation, the pond was cleaned and limed at the rate of 250 kg/ha. After three days of liming, pond was fertilized with urea and Triple super phosphate (TSP) at the rate of 12.50 and 25.0 kg/ha, respectively. Fry of upgraded GIFT strain generation (G_2) were stocked together with the progeny of the founder stock in a pond for communal rearing. Fry of founder population of GIFT strain (Original strain) were marked through cauterization of pelvic fin. For each group, 600 fingerlings were stocked. The initial mean weight of upgraded GIFT strain (G_2) and founder population were 4.81±0.65 and 4.72±0.82g, respectively. After stocking fingerlings were fed with nursery feed contained 28% crude protein at the rate 5-10% of estimated body weight. Fish were sampled at fortnightly intervals to assess the growth as well as for feed adjustment. In the first month, fish were fed at the rate of 10% of estimated body weight and the consecutive months feed ration was reduced to 8, 6 and 4% in the 2nd, 3rd and 4th month, respectively. After four months of rearing, fish were harvested through repeated netting followed by pond drying. The same protocol was practiced in subsequent generations in 2009, 2010, 2011 and 2012 (corresponding to generations G_3, G_4, G_5 and G_6) for growth evaluation.

3. Results

In 2007, a total of 2000 fish (1000 males and 1000 females) of the first generation were harvested and their body weights were measured. General linear model analysis indicated that there was significant difference (P<0.001) in body weight between the two sexes, where the males were substantially heavier than the females (278 *vs*. 156 g) (Table 1).

Table 1: Harvest body weight by gender in G_1

Sex	Number of records	Average weight (g)
Male	1000	277.76±29.77
Female	1000	156.05±30.26

The Estimated Breeding Value (EBV) range for the males and females selected as breeding candidates to produce progeny for the second generation (G_2) were 4.17-9.70 g and 4.24-9.36 g, respectively (Table 2).

Table 2: Breeding values of selected male and female breeders

Sex	Number of animals	Range of breeding values
Male	60	4.17 - 9.70
Female	60	4.24 - 9.36

The body weight data of the upgraded (selected) and founder stock (progeny of non-selected population) were measured at different months. The initial mean weight was 30.23±0.41 and 31.70±0.60 g for the upgraded (selected GIFT) and founder stock, respectively. Month wise sampling data showed that growth rate of the upgraded GIFT strain was always higher than the founder stock. After four months rearing, the final cumulative mean weights were recorded at 168.67±3.51 and 157.33±2.52 g for the selected and founder stock, respectively. Table 3 presents net gain and daily gain for the G_1 and founder stocks. The net gains for weight estimated for the selected GIFT was significantly (P<0.05) higher than that of the founder stock (138.4 *vs*. 125.6 g). The final weight of the selected GIFT was 7.2% higher than that of the founder stock. Survival rate was 100% in both the stocks.

Table 3: Growth rate of the GIFT fish tested in cistern ecology at BFRI (G1 generation)

Population	No of records	Mean net gain (g)	Mean daily gain (g)
Selected GIFT	30	138.43±3.40	1.15±0.03
Founder stock	30	125.50±3.30	1.04±0.02

3.1 Evaluation of growth performances of upgraded GIFT strain (G_2, G_3 G_4, G_5 and G_6)

The harvesting mean weight of upgraded GIFT strain (G_2) and founder GIFT strain were 142±4.18 and 125±3.97 g, respectively. During sampling, the upgraded GIFT strain (G_2) showed higher growth rate than founder GIFT strain in all the events. The mean weight of the upgraded GIFT was 13.60% higher than that of the average GIFT strain. The upgraded GIFT strain showed higher survival rate than founder GIFT which were 91 and 88%, respectively.

The mean weight gain for the generations of G_2, G_3, G_4, G_5 and G_6 and founder stocks (Table 4). The upgraded GIFT of G_3, G_4, G_5 and G_6 were found 23.21 30.30, 35.38 and 39.25% higher growth over founder generation (Fig. 1). In every generation, the upgraded GIFT showed significantly (P<0.05) higher growth than founder stocks. The cumulative gain after six generations of selection for growth performance was 39.3%, averaging 6.54% per generation (or per year). In every generation, upgraded GIFT showed higher survival over founder GIFT.

Table 4: Growth rate of the upgraded GIFT in communal rearing in pond ecology Generation Weight (g)

Generation	Mean weight (g)	
	Upgraded GIFT strain	Founder GIFT strain
G_2	141.60±19.01	125.72±16.54
G_3	151.74±17.29	123.39±17.35
G_4	168.25±23.25	129.17±15.50
G_5	172±3.41	132±4.92
G_6	188±5.21	135±7.88

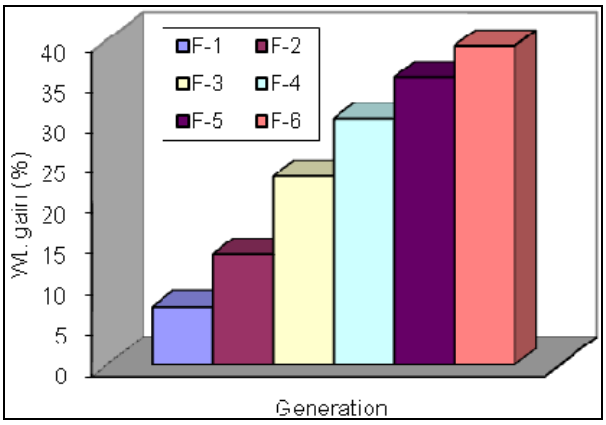

Fig 1: Generation wise percent weight gain in GIFT strain

4. Discussion

The results showed that the upgraded GIFT strain had a significant higher growth than the foundation stock, after six generation of selection. Bangladesh Fisheries Research Institute (BFRI) has initiated stock improvement program for GIFT strain through mass selection in 1998. Through mass selection, G1 generation of GIFT showed 5% higher growth over average GIFT strain, which was introduced from ICLARM (Now WorldFish Center), Philippines in 1994. Subsequent generations (G_2 to G6) were produced in the same manner. Through combined mass selection technology, the G6 generation of GIFT strain achieved 32.7% growth over existing GIFT strain [12]. Initially, the rate of genetic gain in weight of fish was greater up to third generation but it was decreased gradually and continued up to sixth generation. The reason behind such a decrease trend in genetic gain in particularly for body weight might be the accumulation of inbreeding. In another study, reported that the stock improvement of silver barb (*Barbodes gonionotus*) using selective breeding techniques initiated using two wild caught populations from Thailand and Indonesia and an existing local stock from Bangladesh [8]. For producing the parental base population, three unrelated founder stocks were mated through diallele crossing to produce heterogeneous out bred genetic group. A 7.5% genetic gain in growth performance was attained by the F_1 crossbred group over non selected control group. The F_2 and F_3 selected groups' attained 2.3% and 12.1% cumulative weight gain, respectively over two generation.

In the present study, the genetic improvement strategy for enhancing the growth of GIFT strain was re-designed. Now the stock improvement programme is being implemented through family selection protocol instead of mass selection. It was reported that G_1 generation of GIFT which produced by family selection protocol showed 7.20% higher growth over founder population [13]. The present findings indicated that GIFT performed an approximately 39.3% genetic gain after six generations using family selection protocol.

4.1 Dissemination of GIFT germplasm

Due to fast growth and high survival of GIFT strain, the strain is widely cultured throughout the country in both fresh and brackish water as well as in cage culture and rice field ecosystem. Presently, in Bangladesh, over 400 tilapia hatcheries are established in the last couple of years and producing about 5.0 billion fry of tilapia. Aquaculture production in Bangladesh has been dominated by GIFT strain which commenced in 2003. Last nine years (2003 – 2012) a tremendous progress in tilapia farming in Bangladesh. Presently, Tilapia production of Bangladesh is 0.132 million tones. This was due to the development of monosex seed production technology and grows out technique(s) for farming of GIFT tilapia in ponds and cages. Bangladesh should include in the list of top eight tilapia producer states in Asia in near future.

Bangladesh Fisheries Research Institute (BFRI) as a center of excellence has given thrust to produce quality seeds as well as stock improvement program of GIFT strain. In the last year (2012), BFRI distributed 1.0 million fry to 150 tilapia hatcheries. When these GIFT fry attains maturity, the hatchery operators using these fishes as germplasm and they produce millions of monosex fry and sale to the farmers for the production of table size fish. It is expected that in near future, generically improved GIFT strain will be the prime culture species in Bangladesh.

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