ISSN: 2347-5129
(ICV-Poland) Impact Value: 5.62
(GIF) Impact Factor: 0.352
IJFAS 2016; 4(4): 420-424

www.fisheriesjournal.com
Received: 21-05-2016
Accepted: 22-06-2016

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A review on all male mono-sex GIFT seed production by using 17-α methyl testosterone hormone practiced in Bangladesh

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Abstract
The present study was conducted for the review of present method practiced for all male mono-sex GIFT seed production by using 17-α methyl testosterone hormone in Bangladesh. In Bangladesh, all male mono-sex GIFT seed production by using 17-α methyl testosterone hormone is performed from the mid February to November, starting with egg collection. Smaller hatching trays or, jogs were used for the incubation of the collected eggs for 5 days which are eventually followed by incubation in the larger hatching trays for 36 to 48 hours until the yolk sac totally disappeared. The hormone mixed feed (contained 17-α Methyl testosterone (MT) hormone at the rate of 60-70 mg/Kg) was composed of 50% fishmeal, 30% wheat bran and 20% rice bran. The post larva were subjected to hormone mixed powdered feed for the first time after transferring them to transitory tanks and treated there for 5 days. Into the S.R.T. (Sex Reversal Treatment) ponds, further treatment was completed. There they fed frequently with hormone mixed feed for about 21 days and moved further.

Keywords: Tilapia, mono-sex seed, testosterone hormone, GIFT, Bangladesh

Introduction
For a time being, there is a decline in the inland capture fishery due to overexploitation and environmental degradation in Bangladesh and some other countries of Asia. In this context, aquaculture has the potential to increase fish production and to compensate the deterioration in capture fishery to some extent [8]. Among the developing countries for instance Bangladesh, India, Nepal, Pakistan and Sri Lanka have a great potential for the tilapia culture, despite of that the government of these countries had not yet taken any steps. Predominantly in Bangladesh and India, the most attention (either technically or financially) is given to the Indian major carps and Chinese carps and for their development, but main constraint of culture of these species is that they could only be cultured in the freshwater conditions but not in marine or brackish water environment. Tilapia could be feasible to be cultured in freshwater to brackish water or, in semi-saline water [4].

Moreover, they are less expensive and easy to be cultured. In Bangladesh, Tilapia (*Oreochromis niloticus* Linnaeus, 1758 / *Oreochromis mossambicus* Peters, 1852) production was 16,237 metric ton (mt) in 2008-2009, which was 1.78% of the country's total pond production. In cultured pond, it was 13,305 mt, which was 1.5 % of total cultured pond production. In culturable pond, it was 2,868 m.t., which was 7.43 % of total culturable pond production. In derelict pond, it was only 105 mt, which was 2.65 % of total derelict pond production [2, 6, 7]. Mozambique tilapia (*Oreochromis mossambicus* Peters, 1852) was first to be introduced in Bangladesh in 1954 from Thailand and this species did not perform well and proved as weed fish due to its early maturation, copious breeding, ugly ash color appearance and less productivity. In 1974, the Chitralada (Stripped) strain of Nile tilapia (*Oreochromis niloticus* Linnaeus, 1758), a potential farmed species, was being introduced into Bangladesh from Thailand all through UNICEF. After the adoption of Nile Tilapia (*Oreochromis niloticus* Linnaeus, 1758) for aquaculture purposes, it was proven very much successful. The causes behind the success are easier culture technique, sturdy and tolerant of a wide range of environmental conditions. The major constrains of culturing mixed-sex tilapia population are sub-optimal growth and low or variable size range. To evade those problems in the 1970's the all-male fry was produced. Development of hapa-based broodstock management, which allowed for collection of tilapia eggs and yolk sac larvae of an uniform age, proved the key to ensure consistently high (~99%) levels of male fish following the application for 21 days of feed treated with 17-α Methyl testosterone (MT) [9, 10, 12]. This breakthrough occurred because of doctoral research initiated at the Asian Institute of Technology (AIT) in 1984. Genetically Improved Farmed Tilapia (GIFT) strain, a synthetic strain of *Oreochromis niloticus* Linnaeus, 1758, was being set up in July 1994 from Philippines, Under the Dissemination and Evaluation of Genetically Improved Farmed Tilapia in Asia (DEGITA) a project work of World Fish Center.

In Bangladesh, the relative growth and production potential of GIFT and existing Nile tilapia strains (*Oreochromis niloticus* Linnaeus,1758) was evaluated both in the research centers and in the countryside farms. On-station yield of the GIFT was about 57% higher and in on-farm trials, it was 52% higher than the existing local strain and other countries under the DEGITA project results the same. In Bangladesh, the average gain per generation across five generations of selection for growth performance was about 6.7% of body weight. In this genetic selection process the GIFT strain was being developed as much as 31.2% added productive than the former one. The more improved strain of Nile tilapia (*Oreochromis niloticus* Linnaeus, 1758) is more productive than the former one named as the BFRI Super GIFT Strain [3, 4, 11].

The prime objective of the present study was to investigate the existing mono-sex tilapia seed production approach, which includes sex identification of the brood fish, understanding about the size grading of the collected eggs, the technique to prepare stock solution of hormone to produce all male mono-sex *Oreochromis niloticus* Linnaeus, 1758 GIFT seeds and finally the feed administration process, in Bangladesh.

Materials and Methods

The study was conducted in Mymensingh (24º34.5´N, 90º23.5´E) of Bangladesh as it is a very important place in relation to fish farming and research. For the study purpose 10 GIFT tilapia farms were randomly selected. The data was gathered over 4 months from 1st April to 30th July, 2011. For gathering data, combinations of several survey techniques were applied. From appropriate governmental and non-governmental organizations such as Department of Fisheries (DOF), Bangladesh Fisheries Research Institute (BFRI), Agro-3, Fish Hatchery & Culture Farm, secondary data about all male mono-sex GIFT seed production by using 17-α Methyl testosterone hormone were gathered.

The study exertion was performed by following methods; Direct observation: This was performed through the intense observation of every single procedure and segment of the study purposes.

Direct intervention: This was performed through the intervention in the feed preparation, administration etc.

Survey through questionnaire: This was done with the help of a questionnaire and filling up that by consulting with 100 experts, farm owner, manager and other stuffs.

By means of Microsoft Excel software, data from different pertinent sources were coded and recorded into a database system. To assure the accurateness of the data recorded at all steps of the survey, resemblance between preliminary data sheets and the original coding sheets were evaluated; correctness and quality of the data were scrutinized up, edited and coded at the field level.

Results and Discussion

Sex identification of the broodstocks

The sex identification is to distinguish the male GIFT tilapia from the female counterpart (Table 1), this is very much essential because only the female fish would be taken for egg collection purposes and the male fish would be release immediately after collection. Hence, it is an important technique for the egg collection practice. Usually, the external features are well distinguished from male to female (Figure 1). This practice is very important because, if the egg collectors were not well acquainted with the external features of the fishes, there would be unnecessary stress for the male fish through severe handling by collectors. The distinguishing characteristics of the male and female GIFT are listed in the table 1 (Figure 1).

Egg collection technique

In general grown up female releases ovulated eggs at 2–3 weeks intermission under tropical pond circumstances and afterwards fertilized by their male counterpart of the breeding *hapa*. The female carries the fertilized eggs into their mouth. Thus twice or, at least once a week the mouth of the females of the *hapa* should be checked sincerely. The eggs were collected from the mouth of the female fish.

Size grading of the eggs

Size grading of the eggs, much needed and is of great significance, because the eggs of the same maturity or, same age usually reared in the same incubators and eggs of different age of maturity are reared in different incubators (Figure 2 & Table 2). Thus it is of great importance since the fry which have absorbed its yolk sac and turns as first feeding swim up fry are subjected to hormone mixed powdered feed which is the key feature of the study practice.

Table 1: Distinction between female and male GIFT

Characteristics	Female GIFT	Male GIFT
Body shape	Elongated	Deep bodied
Body color	Less attractive than male	More attractive to female
Color of dorsal and caudal fin margin	Grayish	Bright red
Size of genital papilla	Smaller to male	Larger than female

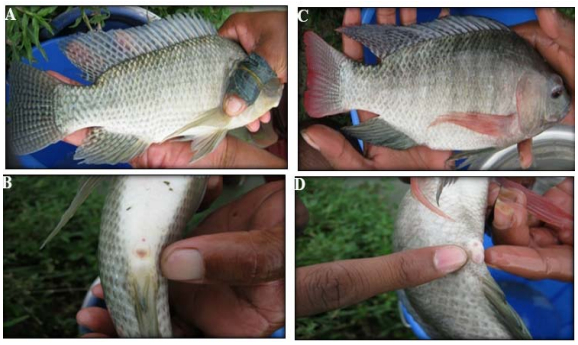


Fig 1: Sex identification of the brood fish: A. GIFT female fish, B. Genital papilla of female GIFT, C. GIFT male fish and D. Genital papilla of male GIFT

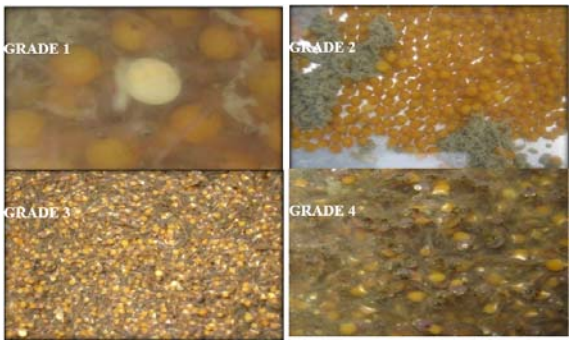


Fig 2: Different size grades of GIFT eggs

Grade 1: Whitish colored ovulated egg, which yet not fertilized.

Grade 2: Fertilized ovulated eggs, those are similar as grade 1 in shape but differ in colors, as it is yellowish color.

Grade 3: These are hatched fry, reddish yellow in color having yolk sac but also have head and tail.

Grade 4: These are well matured than the grade 3, yolk sac either incompletely or totally absorbed.

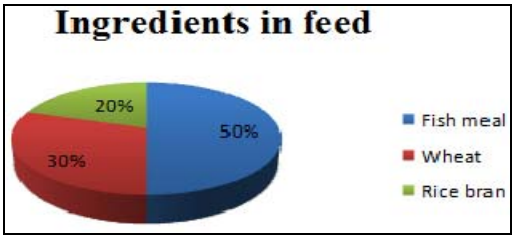
Table 2: Different grades of tilapia eggs based on their appearance, color, size and age

Characteristics	Grade 1	Grade 2	Grade 3	Grade 4
Appearance	Only egg	Fertilized egg	Hatched fry having yolk sac	Yolk sac apparently or totally absorbed
Color	Whitish	Yellow red	Yellowish with silvery appearance	Silvery
Size	-	-	-	0.01g
Age	0	0 to 1 day	1 to 5 days	6 to 9/10 days

Technique to prepare stock solution of hormone (Table 3) for 10 kg hormone mixed feed

Table 3: List of ingredient for preparing the stock solution

Ingredients	Amount
Alcohol (95%)	1000 ml
Powdered hormone	70 mg



Feed formulation

The feed formula (Figure 3 & Table 4) of 10 Kg feed listed below-

Table 4: Ingredients for preparation of 10 Kg feed

Ingredients	Amount	Percentage
Fish meal	5 kg	50
Wheat	3 kg	30
Rice bran	2 kg	20
Multivitamin Solution	1 spoon full	-
Total	10 kg	100

Feed administration

The study practice is based on the direct method of sex reversal by feeding the first feeding swim up fry with hormone mixed powdered feed. Thus, the feed administration is very much significant to ensure proper feed distribution to the target places. The hormone mixed powdered feeds were disseminated both in the S.R.T. *hapas* of the SRT ponds and to the transitory *hapas* of the transitory tanks simply by spreading (Table 5). The brood fish in the breeding *hapas* (Table 5) were fed with hormone free feed at 0.8 to 1% of their body weight twice daily.

Table 5: List of transitory tanks, breeding *hapas* and S.R.T *hapas* showing the life stages of the *Oreochromis niloticus* Linnaeus, 1758 GIFT, days they reared for, feeding rate and feeding frequency

Attributes	Transitory tanks	S.R.T *hapas*	Breeding *hapas*
Life stages	Swim up fry of 8 to 9 days of age	Fry of 13 to 14 days of age	Brood fish
Reared for (in days)	5	21	All the year round but male and female remain segregate in off season, such as in winter
Feeding rate	30 % of body weight	30 % of body weight	3 – 4 % of body weight
Feeding frequency	6 times a day	5 to 6 times a day	2 times a day
Type of feed given	Hormone mixed powdered feed	Hormone mixed powdered feed	Powdered feed without hormone
Total amount of feed given	1.26 Kg/day/tanks	0.225 – 0.27 Kg/day/*hapa*	2.1 – 2.8 Kg/day/*hapa*

Mono-sex tilapia seed

The conclusion of the present study was the mono-sex tilapia seed, which could be further, cultured under extensive, semi-intensive culture condition. The seed could be cultured either monoculture or, polyculture or, even into integrated farming condition along poultry and livestock [8, 11, 12]. It has the capability to culture under varying degree as because it has a wide range of feeding habits. *Oreochromis niloticus* Linnaeus, 1758 is very much able to have wide variety of foodstuff. Tiny phytoplankton and zooplankton and the larger macrophytes are included under its feeding habit. It also grows considerably well on artificial feeds because it respond quite well under intensive culture conditions, where it was fed on complete compound feed. The young fry of tilapia are omnivore and forage for copepods, hydracarina and other various insects both from terrestrial and aquatic origin. The young fish seed of *Oreochromis niloticus* Linnaeus, 1758 GIFT, have a great appearance, which attract the culturist. It has elongated, moderately deep body with black transverse stripes on its silvery outlook. Its size varies from 2.0 to 2.5 cm. In the tropical semi-intensive culture condition, it could be 150 to 250g in weight in just 4 to 6 months [4, 5].

Hormonal sex reversal has been reported in a plenty of families of fish including Cichlidae, Cyprinidae, Ictaluridae, Percidae, Poeciliidae, Salmonidae etc. Since the gonadal differentiation of cichlids occurs early in the life history, so it is very much effective for them. Tilapia species, which are, mouth brooders for example *Oreochromis niloticus* Linnaeus, 1758 shows successful sex reversal instead of the substrate spawners for instance, *Tilapia zilli*. Male tilapia is preferred as per rapid growth rate and redirects less energy in the reproductive purposes. Various chemicals used for regulating sexual development in the tilapia. Regarding the hormonal dosage applied in livestock production, or human medicine, the MT used for tilapia farming is very small. In tilapia farming, only the early fry stages are introduced with hormonal dosages, typically less than 0.02 mg for each tilapia in total. Furthermore, after the ending of hormone treatment they usually reared at least for another five months until they attain marketable size. With a view to produce mono-sex, tilapia stocks to overcome the extensive problem with the copious reproduction of tilapia a plenty of hormones and various treatment methods were experienced [4, 9, 10, 12].

Summary of the whole procedure

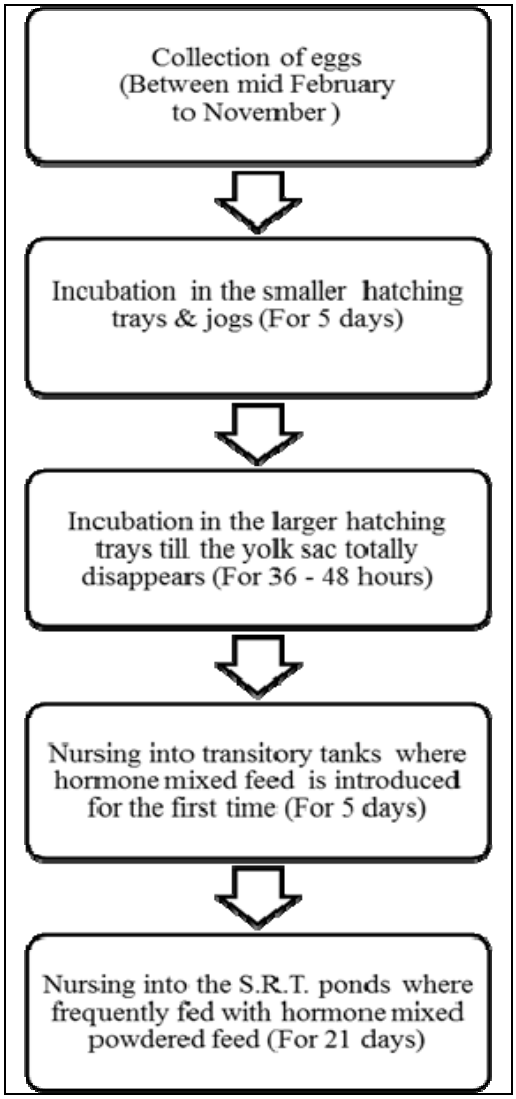


Fig 7: Flow chart of the entire procedure of mono-sex tilapia seed production starting with egg collection and ending into the SRT ponds

Almost all the treated fish develop as males morphologically, by feeding a little amount of hormone to tilapia fry before and in the course of sexual differentiation, and thus the probability of the stock to reproduce discarded. This method has additional benefit in the way because male individual of tilapia grows much faster than its counterpart and mono-sex male tilapia has larger and more uniform size than mixed-sex tilapia. The uniformity and larger size of MT treated tilapia makes them highly apt for export, notably to supply the fast growing demand for fresh and frozen fillets. Those characteristics stated earlier are best suited for Nile tilapia (*Oreochromis niloticus,* Linnaeus, 1758); foremost tilapia species farmed commercially worldwide. Body weight distinctions between male and female *Oreochromis niloticus* are significantly visible after 100 days and male to female size superiority continues to increase with age [5].

For the present study, all the collected eggs were separated to size grade (Table 2 & 5, Figure 2) wise in order to observing individual growth as this step is of prime importance because the fry in this stage have almost absorbed its yolk sack and ready to fed with exogenous food (Table 2 & 5, Figure 2). The fry in this stage fed with hormone mixed feed, because, phenotypically fries are "totipotent [1, 9, 10, 12] (capable to develop either sexual phenotype)" and its sex can be changed through hormone administration is very early life stage and this is known as direct method of sex reversal.

The androgen treatment efficacy generally rest on the hormone dosages. Conversely, the high dosages might be resulted as gonadal growth reduction, gonadal intersexuality and feminization. Modification in the spermatogenesis that is to say inhibition of spermiation, depleted potency and scanty generative implementation in numerous teleostean species treated with synthetic androgens has also been reported. MT dosage should be restricted to maximum of 50 mg/kg [4, 5], but for this study purposes the dosage used was 60-70 mg/Kg as the producers are dubious about the purity of the readily available hormone of the market. The success of the sex reversal predominantly depends on the suitable and correct hormone dosage.

The amount of MT applied universally in aquaculture practice yet not accounted, but it could be concluded that the vast majority of tilapia products marketed globally (Including almost all tilapia fillets) comes from MT treated ones. MT treated fry is truly the easiest and trustworthy method to produce all male tilapia, eventually grows steadily to a larger or more uniform size instead of all female or mixed sex stocks [6, 9, 10, 12]. The method is mostly applicable to Nile tilapia *Oreochromis niloticus* Linnaeus, 1758, most extensively cultured species throughout the world and therefore the MT treatment came across as the standard method to produce all male tilapia population.

MT is synthetically produced hormone, which shows resemble largely to the naturally producing testosterone hormone. The legitimacy of using the hormone in aquaculture may differ from one country to other. Form the available scientific proofs it could be concluded that tilapia treated with MT has no ill effects to human health when applied with recommended dosages in the earlier stages of life. The amount of MT consumed by tilapia fry when treating is insignificant in contrast to the level of testosterone produced by human and consumed through other foodstuffs for instance meat and dairy products [9, 10, 12].

Moreover, the studies show that virtually the residual period of this hormone is 3 weeks after removal from the diet.

Tilapia readily defecates ingested hormone, the level falls to less than 1% within 100 hours after withdrawing MT. As a result MT is not detectable in adults whom have passed through growth period of no less than five months while reaching marketable size [10, 12].

Moreover, yet there is no complaining about health effects of workers at tilapia farms where MT is be used. Nonetheless, all the possible recommended procedure for dealing might be applicable as routine precautions.

Conclusion

The study work starts with the collection of eggs from the mid February and ends at November each year and the fry is frequently fed with hormone mixed feed for about 21 days. The hormone mixed feed contained 17-α Methyl testosterone (MT) hormone at the rate of 60-70 mg/Kg. The production of mono-sex male tilapia using androgen is very much efficient. The suitability of this method is that it does not require any portion of the production to be cast off as in the manual selection, or so as to, two separate stocks of fish are maintained as in hybridization. Even though a plenty of hormones have been using for sex reversal purposes, methyl

testosterone is most frequently used androgens. Dosage rate and treatment period may vary according to the environs and skill of the producer.

Nonetheless very little information is known regarding the environmental impacts of discharging the waste water from tilapia hatchery be utilize MT treatment, the main reason behind is, most of the research till now is concerned only with the impact of estrogens and their anti-androgen on natural fish and other animals, however, its primarily established that the 17-α methyl testosterone hormone is broken down when exposed in the sun light of the natural water sources. However, the amount be intruding to the environment are very lower than from those released from agricultural wastes and domestic sewage. Although steroid hormones like MT are immersed swiftly onto sediments and alike. The gravel and sand filters, or bio-filters as well as wetlands rapidly can remove hormones from water within 24 hours. As aquaculture enduring to an ancillary helping of the world's fisheries products, tilapia culture will play more significantly. Sex reversal will stay the industry orthodox for controlling reproduction if tilapias.

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