

A Novel Technique to produce all Male Tilapia – An efficient solution to a potential problem

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ABSTRACT

Early sex maturation and frequent spawning are management challenges when controlling over populated, stunted tilapia population in Sri Lankan aquatic systems. Maintenance of only male tilapia population is the most appropriate mechanism for controlling them in aquaculture facilities. Hormonal sex reversal is the most commonly used procedure used to develop male tilapia. Exogenous steroids given during the gonadal development period (labile period) can control the phenotype overriding the expression of the genotypically determined sex. This study aimed to use an immersion technique with 17- α Methyl testosterone (17 α -MT) hormone at high temperature (34 °C) in order to produce all male tilapia population in breeding stations using an effective and environmental friendly method.

Experimental results showed that there is a significant difference ($p < 0.05$) between all male tilapia population observed in hormone immersion treatment done at high temperature (100% male) compared to the fish observed from hormone alone treated fish (89% male), and the control (50% male). Our results proved that tilapia fry treated with 1.5 mg/L concentration of 17- α MT hormone at high temperature (34 °C) exposure during its labile period produced an all male population. Application of this technique in fish breeding stations can produce all male tilapia in an environmentally safe manner with a low cost of production. Further, this immersion technique can be used as a reliable method to produce all male tilapia and for introduction into water bodies where tilapia are presently over populated. Thus we expect to manage tilapia population in an environmentally sound manner while minimizing their invasiveness in water bodies in Sri Lanka.

Key Words: Tilapia, *Oreochromis niloticus*, all male populations, hormonal sex reversal, Sri Lanka

INTRODUCTION

In Sri Lanka, Tilapia (*Oreochromis niloticus*) has higher consumer attraction than other freshwater fish in both rural and urban areas. Nile Tilapia was introduced to Sri Lanka in 1950s to further increase productivity of freshwater fisheries. In commercial pond fish culture, tilapia needs successful culture methods to cater to the demand, in addition to harvested tilapia from the island's aquatic systems.

One of the main problems of Nile Tilapia culture is their early sexual maturation (4-5 months old) which results in successive spawning during the growing season. This unwanted reproduction usually leads to crowded conditions in the ponds and consequently reduces growth (Varadaraj & Pandian, 1987). This situation also applies to aquaculture facilities in Sri Lanka where early sex maturation and frequent spawning are management challenges when controlling over populated, stunted tilapia populations. Males tilapia grow bigger and faster than females. Therefore, as a remedy, all-male population are preferred to avoid unwanted spawning in a production unit as well as giving higher productivity.

A recent controversy has emerged in Sri Lanka over the inclusion of Nile Tilapia as a potential invasive species (Marambe *et al.*, 2011) that can threaten native biodiversity of the country's rivers, streams and reservoirs. This species plays a very important role in contributing to availability of fish protein especially in rural areas. Tilapia is the dominant species accounting for over 60 percent of inland fish populations. Sri Lanka does not import inland fish, and thus relies on local production. All in all, programs of the government and non-government organizations have supported the development of culture-based fisheries in perennial reservoirs through stocking of fish fingerlings of suitable species. We believe that sex reversed tilapia can play an important role in preventing of rapid expansion of their population due to unwanted breeding.

Several methods have been used to increase the percentage of males in cultured tilapia populations. Control of unwanted reproduction in cultured tilapia population is possible through mono-sex culture, which may be achieved by various method including manual sexing, hybridization, genetic manipulation and hormonal sex reversal to produce all male tilapia populations. The major disadvantages of the first method are human error in sexing and of course the wasting of females. The main disadvantage of the hybridization method are difficulty in maintaining pure parental stock that consistently produce 100 % male offspring (Pruginin *et al.*, 1975), as well as the poor spawning success (Lee 1979). All male production by genetic manipulation requires a lot of skill, infrastructure and the results are not always consistent. Sex reversal and genetic manipulation (YY) males do not always yield 100% males because sex determination in tilapia is not only controlled by sex chromosomes and hormones as some studies have revealed that temperature plays a role in sex determination as well as some genes in autosomes (Phelps and Popma, 2000).

Most commonly used methods to produce all male tilapia are the hormonal sex reversal (Gale *et al.*, 1997) of fry by feeding or immersion in hormone containing solution. Administering androgenic hormones during labile period can reverse the entire or majority of the fry population into effective males. This is done by submerging the fry in hormone solution or injecting hormones. Immersion method of hormonal sex reversal needs shorter time period than other methods mentioned. The steroid hormones for sex reversal used at present are: 17 α Methyl Testosterone (MT), Ethynyl Testosterone (ET), Methyl Dihydro Testosterone (MDHT) and Tronbolone Acetate (TBA). In immersion protocol, fry are immersed in hormone solution at 11th and 13th days of post fertilization. Time duration for immersion in hormone solution varies from 2-3 hours (Bart *et al.*, 2003).

This study aims to demonstrate the results of the immersion technique with 17 α -MT hormone during labile period of tilapia fry as an efficient and environmental friendly mechanism to produce all male tilapia than the feeding technique presently used to produce all male tilapia in Sri Lanka.

OBJECTIVES

Objectives of this study were to produce all male tilapia using a hormonal immersion technique for the stock enhancement program of all male population and to manage the stunted and overcrowded tilapia populations in Sri Lankan aquaculture facilities in a more economically viable and environmental friendly manner.

MATERIALS AND METHODS

a. Experimental site and treatments

Experiments were conducted at National Aquaculture Development Authority (NAQDA)-Dambulla and CIC Agribusinesses- Pelwehera in North Central Province. Nile Tilapia (*Oreochromis niloticus*) fry at 11th and 13th days of post fertilization (dpf) were used. Immersion technique using 17 α -MT hormone 1.5 mg/L concentrations were used as the treatments and fresh water with 98w/w Ethanol (without hormones) was used as the control.

b. Brood stock selection and management

Tilapia broodstock was maintained at 1: 3 ratio of male: female at NAQDA, Dambulla. Disease free, healthy, and well developed 5 male and 15 female *Oreochromis niloticus* were selected as brood stock fish. The average body weight was 250 – 350g. They were separated in to 20'x10' (length and width) cemented broodstock tank. Water level of the tank was maintained at 1.5 feet depth. Feeding was done three times per day as 3% of body weight equivalent feed (Charoen Pokphand-Thailand) per day which contained 30% Crude Protein. After every 3 days female fish were caught and mouths were checked for presence of eggs.

c. Selection of experimental fish

Two thousand and four hundred (2400) 3rd eyed stage eggs (3 dpf) were collected from the mouth of female tilapia during the 3rd day of post fertilization during mouth incubation period. Collected 3rd eyed stage eggs were transferred into 12", 18" and 3" hatching trays which were placed close to the broodstock tanks for artificial incubation. Incubation was conducted (2-3 days) until hatchlings were emerged from fertilized eggs. A constant water flow was supplied for gentle circulation of eggs to provide sufficient oxygen, prevent eggs being deposited on

the bottom of the trays and removal of nitrogenous excreta. A total of 2000 hatchlings were obtained and 1800 fry from these were subjected to the experimental conditions at 600 fry per each treatment.

d. Hormone treatment and experimental process

Three treatment conditions were established including the control with three replicates (200 fry/replicate) for each. Once the *Tilapia* fry were at 11th and 13th day of post fertilization (dpf) they were immersed for a 3h time period each day in a concentration of 1.5 mg/L MT hormone solutions at 34°C water temperatures as treatment-1, with 3h 1.5 mg/L MT hormone immersion for treatment-2 and neither hormone nor high temperature exposure as the control. The 34°C, high temperature exposure provided for treatment-1 fry was done only during 10th to 18th dpf. Fry density was maintained at 33fry/L during immersion treatment for all treatments and the control.

Then treated fry were placed in two locations (NAQDA-Dambulla and CIC-Pelwehera) for growing in nine separated *hapa* (Netted boxed shape fish culture cage) maintaining three replicate for each treatment with stocking density of 100 fry/*hapa* (size 5'X10'). Feeding (diet with 30% Crude Protein) was done according to body weight while maintaining similar environmental conditions at both locations. Water quality parameters such as dissolved oxygen and temperature were maintained optimum levels while ammonia and nitrite levels were maintained at minimum levels possible throughout the culture period. At 14 weeks of age, sex identification was done for all experimental fish including the control by externally observing genital organs (one hole with penis as the male or three holes as the female) through application of methylene blue and observation of the shape of the end of the dorsal fin (pointed- male or round shape-female).

e. Final harvesting and gonadal identification

Histological analysis was performed once fish reached the age of 21 weeks. Fish sex identification was done for all experimental fish including the control by microscopically observing gonadal tissues using gonadal squash method using Aceto-carmine. The gonad tissues were removed using scissors and forceps, and were placed on glass slides. A few drops of Aceto-carmine was added, covered by microscope cover slips and pressed down firmly to obtain a good stain. Gonads were identified under a light microscope using magnification x40. The fish with gonads containing oocytes were classified as female and fish with gonads without oocytes and having only spermatocytes were classified as males. In a gonadal squash of an ovary, eggs of various sizes will be evident throughout the gonad (Figure 1). It is possible to focus the microscope to view the nucleus of an egg as well. Testicular tissue does not show such features. Lobes of the testis will be apparent but other structures are not distinct (Figure 2). Connective tissue, oviduct or sperm duct may also be apparent.

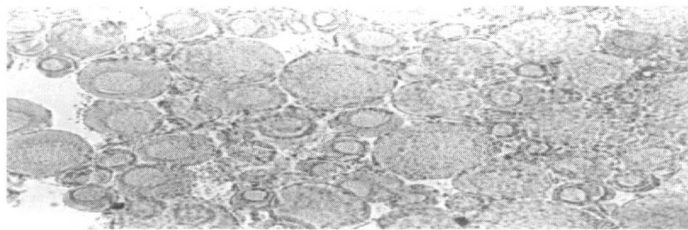


Figure 1: Tilapia ovary with individual eggs as it appears in a gonadal squash

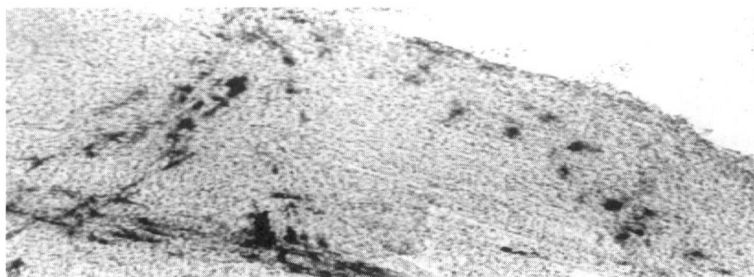


Figure 2: Testicular tissue of tilapia as it appears in a gonadal squash.

f. Statistical analysis

Experiment was conducted in a completely randomized design and data were analyzed using SAS with one-way ANOVA.

RESULTS AND DISCUSSION

Experimental results (Figure 3 & Figure 4) showed that there is a significant difference ($p < 0.05$) between tilapia population (100% male) observed in hormone immersion treatment done at high temperature (Treatment-1) compared with the populations of fish observed from hormone alone (Treatment-2) (89% male) and the control (50% male), respectively. Further, experimental results showed that there is a significant difference ($p < 0.05$) in male population in the control (CR) and other two treatments where hormone was applied.

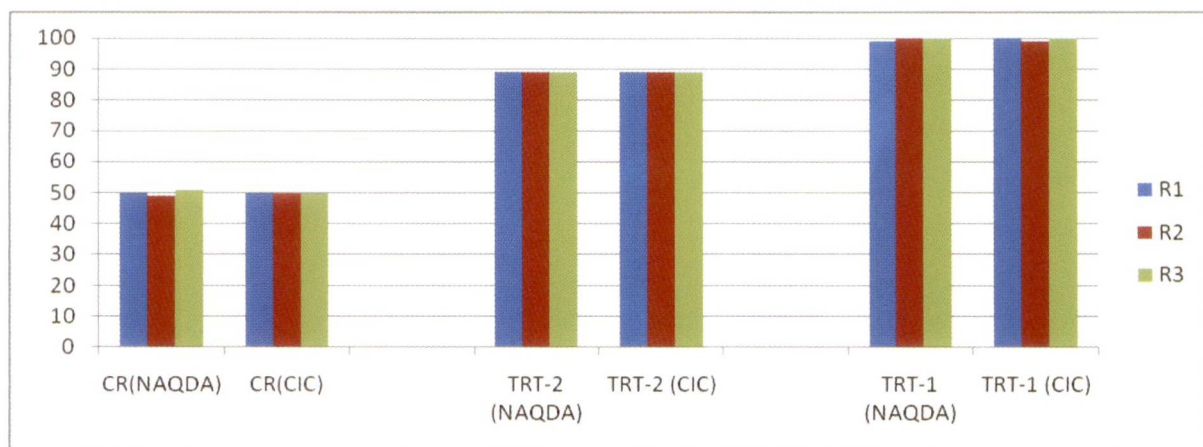


Figure 3: Percentage of male tilapia observed in two different culture locations (NAQDA-Dambulla & CIC Agri businessess- Pelwehera) under three different treatment combinations with three replicates each.

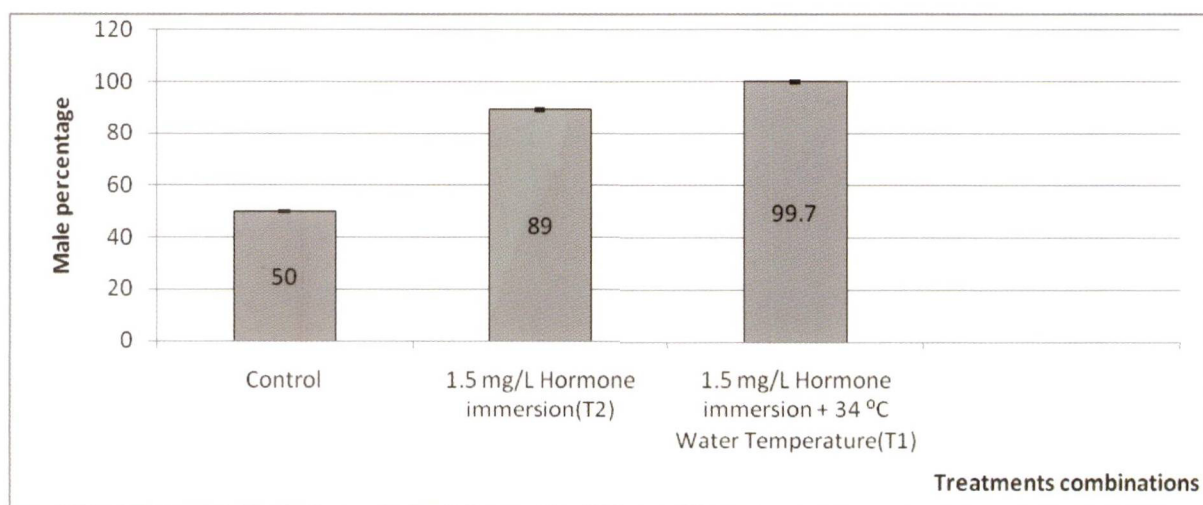


Figure 4: Percentage of male tilapia observed according with treatment combinations.

According to the results obtain edit has been demonstrated that 99.7% male population can be achieved by using the immersion of fry in 1.5 mg/L hormone concentration for two days (11th and 13th dpf) while exposing them to water temperature of 34 °C during the 10th to 18th days of post fertilization. Previous studies conducted at the same locations by Athauda & Kumara. (2015) and Kulathilaka & Athauda (2013) also reported that populations with 97.34% and 98.03% of male tilapia were obtained with 1 mg/L and 1.5 mg/L MT concentration with exposure to water temperature of 36 °C. Results of this experiment proved that when the tilapia fry treated with 1.5 mg/L concentration of 17 α - MT hormone with exposure to water temperature of 34 °C during its labile period produces the all-male population without mortality.

CONCLUSIONS AND RECOMMENDATIONS

Application of hormonal immersion technique in fish breeding stations where the feeding technique is currently being practiced can produce 100% all male tilapia in environmentally safer manner at low cost of production.

The project was planned with the aim to determine the optimal dosage and the most effective time period to provide high temperature for immersion technique of the androgen 17- α methyl testosterone (17 α -MT) for effective sex reversal of Nile tilapia. A population of 99.7% male population can be achieved by using 1.5 mg/L hormone concentration and 34°C temperature at 10th to 18th days of post fertilization. It revealed that it is sufficient to give high temperature at that time period with recommended hormone concentrations in order to achieve the highest male tilapia population. While the 1.5 mg/L hormone concentration increases the productivity and efficiency of the treatment, the short time period of temperature exposure can be helped to reduce the cost of production of all male tilapia.

Introduction of all male tilapia populations into culture-based fisheries and pond fish aquaculture now being practiced in Sri Lanka will increase the fish productivity. Further, it eliminates the stunting and overcrowding problems that occur in present cultured tilapia populations in aquaculture facilities. Ultimately it improves the living standard of fishing community and economic and production status of the country. In addition to above, the potential environmental damage that could be caused by presently overcrowded populations can be controlled while using tilapia as an economically important exotic fish species for enhancing inland fish production in Sri Lanka. Control of unwanted breeding of tilapia in culture facility can be done by stocking of all male (100%) tilapia populations produced through technique as a way to reduce the threats to biodiversity as this species has been included as a potential invasive species in the list of IAS of Sri Lanka.

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