# Masculinization of Nile Tilapia (*Oreochromis niloticus*) through Immersion in 17α-Methyltestosterone or 17α-Methyldihydrotestosterone

**Interim Work Plan, Africa Study 2**

**William L. Gale, Martin S. Fitzpatrick, and Carl B. Schreck**
**Oregon Cooperative Fishery Research Unit**
**Department of Fisheries and Wildlife**
**Oregon State University**
**Corvallis, Oregon, USA**

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## Introduction

All-male populations are used in tilapia aquaculture because mixed sex populations often undergo precocious maturation, which shunts energy to gonadal rather than somatic growth, and early reproduction, which leads to the harvest of many unmarketable fry. Furthermore, all-male populations are desirable, because males grow larger than females.

One of the most common techniques for producing mono-sex populations is steroid-induced sex inversion. This involves the administration of synthetic androgens (to produce male populations) or estrogens (to produce female populations). Several methods of steroid administration are possible, including injection, microencapsulation, feeding of steroid, and immersion of fry in steroid solutions. The latter two are non-invasive and therefore, the most practical for application to aquaculture.

The use of steroid-treated feeds for the production of all-male populations is widespread in tilapia aquaculture. Conversely, the use of immersion techniques is not fully developed for practical use. Torrans et al., (1988) successfully masculinized blue tilapia (*O. aureus*) by immersion in the synthetic androgen mibolerone (MB; 17-hydroxy-7,17-dimethylestr-4-en-3-one). The optimum conditions for treatment were a five-week immersion period at 600 µg /l, with steroid replacement weekly. Pandian and Varadaraj (1987) masculinized Mozambique tilapia (*O. mossambicus*) by immersion in 17α-methyl-5-androsten-3ß-17β-diol (5 or 10 µg/l). The immersion period lasted 10 days, beginning at 10 days post fertilization. Although the authors reported 100% masculinization, detailed information regarding temperature, type of culture system used, fish density, and frequency of water exchange during the immersion period was not included. These omissions make the replication and future application of this research difficult.

In salmonid aquaculture, a short term immersion of fry in a solution of 400 µg 17α-methyltestosterone (MT; 17-hydroxy-17-methylandrost-4-en-3-one)/l for a period of 2 hrs successfully produces all-male populations (Piferrer and Donaldson, 1989; Feist et al., 1995). Methyltestosterone is one of the most commonly used sex inverting agents, but is susceptible to aromatization and has been associated with paradoxical feminization in coho salmon (*Oncorhynchus kisutch*; Piferrer and Donaldson, 1991). Paradoxical feminization can be avoided by use of a nonaromatizable androgen such as 17α-methyldihydrotestosterone (Piferrer et al., 1993).

The objective of this research was to develop a short term immersion procedure for the masculinization of Nile tilapia (*O. niloticus*). Compared to feeding methods, immersion reduces human handling of steroid and provides more uniform exposure of fish to steroid. Two synthetic androgens were used, MT and 17α-methyldihydrotestosterone (MDHT; 17ß-hydroxy-17-methyl-5α-andro-stan-3-one). Methyldihydrotestosterone is also known by the trade name mestanolone.

## Materials and Methods

Steroids were obtained from Sigma Chemical Company (St. Louis, Missouri) and stored in stock solutions of HPLC grade methanol (10 mg/ml). Breeding families (one male to three females) were placed in 208-L aquaria. The temperature was maintained at 28-30°C. Breeding activity was

Table 1. Sex distribution, mortality data, and sample size (n) from experiment one (EX I) and two (EX II). Group abbreviations are as given in Fig. 1.

| Group | EXI | | | | EXII | | | |
|---|---|---|---|---|---|---|---|---|
| | males | females | Mortality (%) | n | males | females | Mortality (%) | n |
| MT-100 | 30 | 11 | 58 | 41 | 12 | 17 | 26 | 29 |
| MT-500 | 16 | 15 | 46 | 31 | 16 | 14 | 64 | 30 |
| MDHT-100 | 33 | 13 | 53 | 46 | 15 | 14 | 33 | 29 |
| MDHT-500 | 36 | 0 | 63 | 36 | 31 | 2 | 33 | 33 |
| ETH | 18 | 23 | 59 | 41 | 14 | 18 | 22 | 32 |
| CTL | 7 | 12 | 81 | 19 | 17 | 34 | 35 | 51 |
| FED | 23 | 2 | 62 | 25 | --- | --- | --- | --- |

monitored daily. Once breeding occurred, the other fish were removed and the brooding female left to incubate the progeny. At 10 days post fertilization (DPF), fry were removed from the female and assigned to experimental groups (n=100/group). Each group was housed in a separate 3.8-L glass jars with 3 L of fresh water. The water was maintained at 28 ± 2°C under constant aeration. Treatment consisted of a 3 hr immersion on 10 and 13 DPF. Steroid was evaporated under $N_2$ (g) and delivered in 0.5 ml of ethanol. Steroid was allowed to mix by aeration for 30 min before addition of fry. Fry were immersed in MT or MDHT at 100 or 500 µg/l (MT-100, MT-500, MDHT-100, MDHT-500). Control groups included the following: immersion in water and ethanol vehicle (ETH), an immersion in water alone (CTL), and water immersion followed by feeding of MT-treated diet (FED; 60 mg/kg) from 10 to 30 DPF. After each immersion, the fry were collected and placed in new jars that contained fresh water. The first experiment (EX I) was replicated (EX II) with omission of the FED group. In EX I, the groups were held in the jars (3.8 L) until the end of the feeding treatment period (30 DPF). In EX II the groups were held in the 3.8-L jars only until after the 13 DPF immersion. The groups in both experiments were transferred into separate 20-L chambers for grow-out in a recirculating system. Water temperature in the grow-out system was maintained at 28 ± 2°C. At 100 DPF, sex ratios were determined by examination of *in situ* (40X) and squash (100X) preparations after aceto-iron hematoxylin (Wittman, 1962) staining. The weights and total lengths of sampled fish were recorded (EX II) at this time.

Sex ratio data were analyzed using the chi-square test ($a<0.05$; Zar, 1984). The CTL and ETH groups were not significantly different, and were pooled for comparison to other groups. The mean final weights of sampled fish from EX II were analyzed for differences between groups using one-way ANOVA ($a<0.05$). Mortality data were analyzed using the chi-square test ($a<0.05$; Zar, 1984).

## Results

Immersion in MDHT at 500 µg/l resulted in 100 (EX I) and 94 (EX II) percent male populations (Fig. 1). In EX 1, MT and MDHT treatments at 100 µg/l resulted in significant skewing of the sex distribution toward males (73 and 72 percent male, respectively); however in EX II, the proportion of males were not significantly different from controls (Table 1). Methyltestosterone at 500 µg/l had no masculinizing effect in either experiment. The MT feeding treatment resulted in 92 percent males (EX I).

Mortality was not significantly different between treatment groups in EX I (excluding CTL). The higher mortality observed in the CTL group in EX I was likely due to a clogged inlet that restricted water flow for about 24 hr. The ethanol treated and control groups in EX II, had significantly different mortality; however, the remaining groups were not different from either ETH or CTL. The MT-500 group suffered higher mortality due to cannibalization by an adult fish that jumped from a neighboring tank. No significant differences between treatments in weights or lengths were detected in EX II.

## Discussion

Immersion treatment on 10 and 13 DPF with MDHT at a concentration of 500 µg/l successfully masculinized Nile tilapia. Conversely, MT at similar levels did not significantly alter the sex ratio. Lack of an effect in the MT treatment (500 µg/l) may be due to conversion of MT to a less active form, or simply to a higher rate of clearance from the body than MDHT. Another possible explanation for the differing effects of the two steroids is that MDHT is a more potent masculinizing agent than MT. Piferrer et al., (1993) found that MDHT was twice as potent as MT in masculinizing female chinook salmon (*Oncorhynchus tshawytscha*).

Administration of steroid by incorporation in feed has a long history of use (for reviews see Schreck, 1974 and Hunter and Donaldson, 1983). Steroid is dissolved in a carrier (*e.g.*, ethanol or acetone), uniformly mixed with feed, and allowed to dry before use. Tilapia fry are fed for 21 to 35 days, beginning somewhere between 10 and 14 DPF. Although this technique results in successful sex inversion, certain inefficiencies are cause for concern. The dose received by an individual fish is likely variable, due to differences in body size and social status. The culturist must then accept partial or incomplete sex inversion or increase the treatment dose to beyond the optimal requirement to achieve 100% sex inversion. Furthermore, the long period of treatment employed by typical feeding methods results in human handling of anabolic steroid three to five times daily for up to 35 days. This degree of handling presents an added risk to the aquaculture worker, given the tumorigenic and teratogenic effects of anabolic androgenic steroids (Lewis and Sweet, 1993). This risk is easily mitigated by the establishment of proper handling procedures. However, often these precautions are not properly implemented. For instance, in developing countries where much of the worldwide tilapia production occurs, disposable rubber gloves for the handling of treated feed may be either unavailable or too expensive to be practical. Furthermore, it is common in developing countries for workers with little or no protective clothing (*e.g.*, rubber waders) to wade in ponds containing dissolved steroid while grading or sampling treated fish. Therefore, techniques that minimize worker exposure to anabolic steroid, but are as (or more) effective as feeding treatments need to be established.

Torrans et al., (1988) described an immersion technique for masculinization of blue tilapia, using the synthetic androgen MB. Steroid solutions were exchanged weekly over the five-week treatment period; thus, steroids were handled directly only five times. However, the total treatment period remains similar to feeding methods and requires that fry be held in tanks during treatment. The technique described in our study consisting of immersion in MDHT decreases the treatment period, thereby reducing worker exposure while still achieving nearly complete masculinization.

Table 2. Average (±SE) weight, total length, and sample size (n) from experiment two. Data collected after sampling (100 DPF) for determination of sex distribution. Group abbreviations are the same as given in Fig. 1.

| Group | Average Weight (g) | Average Length (mm) | n |
|---|---|---|---|
| MT-100 | 2.77±0.21 | 41.7±1.2 | 29 |
| MT-500 | 3.45±0.21 | 44.4±1.0 | 30 |
| MDHT-100 | 2.95±0.30 | 41.7±1.4 | 29 |
| MDHT-500 | 3.29±0.22 | 43.7±1.1 | 33 |
| ETH | 2.97±0.26 | 42.5±1.3 | 29 |
| CTL | 3.17±0.23 | 42.2±1.0 | 50 |

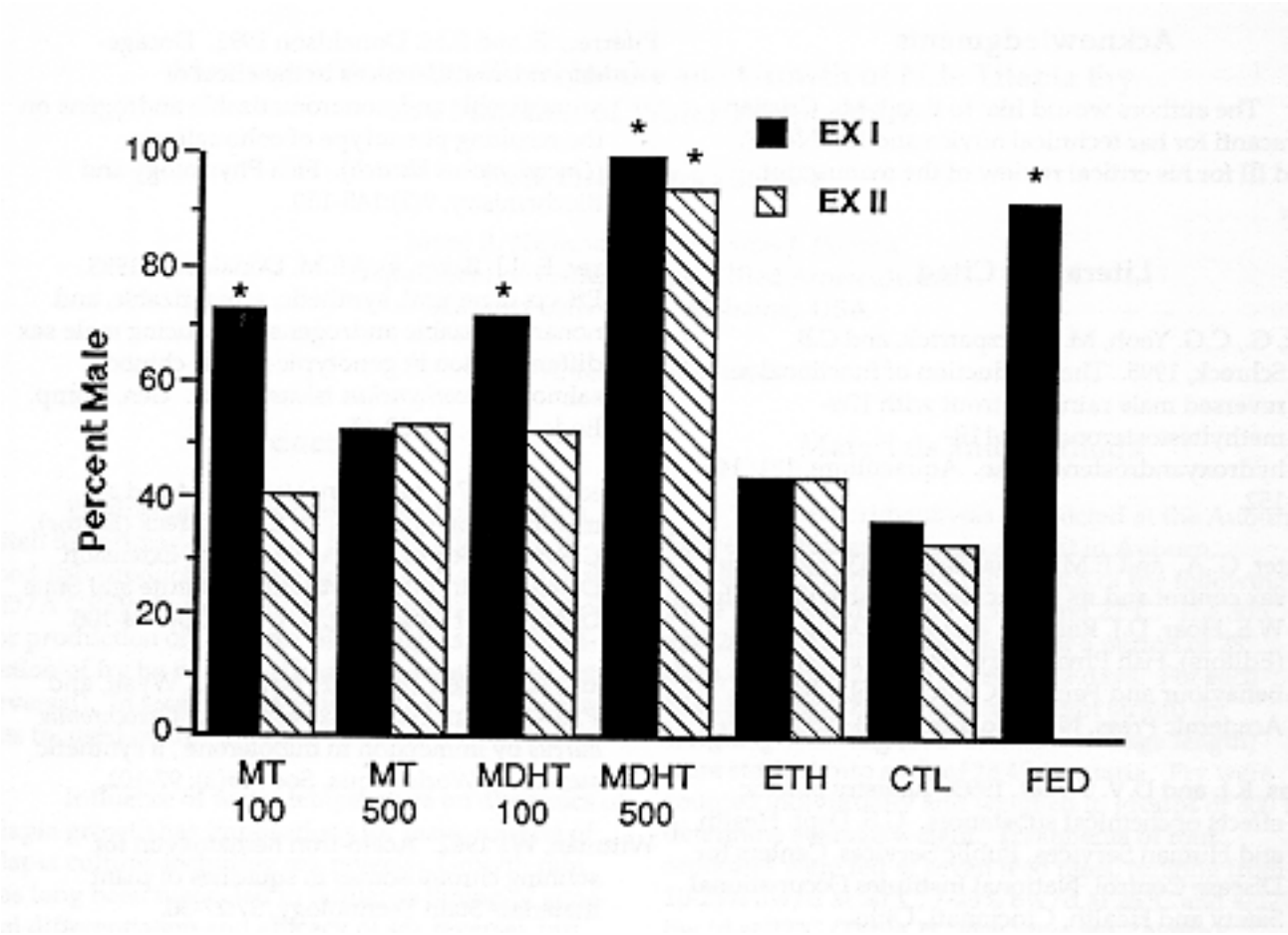


Figure 1. Percent males in each group for experiments one (EX I) and two (EX II). Group designations are as follows: immersion treatment in 100 or 500 µg 17α-methyltestosterone/l (MT 100, MT 500), immersion in 100 or 500µg 17α-methyldihydrotestosterone/l (MDHT 100, MDHT 500), immersion in ethanol vehicle (ETH), immersion in water alone (CTL), and methyltestosterone feeding treatment (FED) from 10-30 DPF (60 mg/kg feed). Asterisks indicate significant (from chi square test; $a \leq 0.05$) differences in proportion of males from the pooled control (ETH and CTL) group. Sample sizes are given in Table 1.

Masculinization of Nile tilapia by immersion in MDHT may provide a practical alternative to the use of steroid-treated feed. Shortening the period of treatment and amount of handling required reduces the risk of worker exposure to anabolic steroids. Furthermore, fish are confined for only three days during the treatment procedure, and our results suggest that MDHT immersion does not affect mortality or growth. This technique shows much promise, but further evaluation is needed before application in large scale aquaculture operations.

## Anticipated Benefits

Immersion in 17α-Methyldihydrotestosterone may provide an alternative method of masculinization to dietary steroid treatment. Immersion provides a substantially reduced treatment period and increased control of worker and environmental exposure to anabolic steroids. Furthermore, fry exposure to steroid may be more uniform and more efficient using immersion than feeding techniques.

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