Sex reversal in Nile tilapia: Is it possible to produce all male stocks through immersion in androgens?

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One of the primary tilapia species cultured around the world is the Nile tilapia (*Oreochromis niloticus*). Usually, monosex male stocks of Nile tilapia are desirable, because cultivation of mixed sex tilapia leads to overcrowding of the tanks and consequently poor growth rates. The negative effects of mixed sex culture are related to some reproductive biology characteristics of the Nile tilapia. We will briefly explain some of these, to help you to understand why monosex male stocks are preferable. In this species, males and females can reach sexual maturation approximately 3-5 months after hatching and fish weighing between 20-40 g are usually ready to spawn. Males and females can spawn throughout the year in water temperatures above 23° C. Females can spawn at every 20-40 days intervals and each one can produce between 4-8 eggs per gram of body weight. So, a female weighing 30 g could generate, every 40 days, 120-240 new alevins. In addition, females incubate the eggs orally, which substantially increase the survival of the offspring (Figure 1 A-D). Females may keep the alevins in their mouths up to 15 days post hatching, releasing them when they are able to swim and feed by themselves. During this period females do not eat and therefore have poor growth. New offspring released into the tank will increase competition for space, water quality, and food. Taking into account that the grow-out period of Nile tilapia is between 6 to 12 months, it is quite easy to realize why mixed sex culture is undesirable. Culture of all male stocks eliminates these negative aspects. Males also grow faster than females, inasmuch as males do not need to allocate any of their energy reserves for the production of eggs.

Sex control techniques in tilapia culture have been an important tool to produce male monosex stocks, which has been key to the development of this aquaculture industry. These techniques can produce monosex fish stocks with the desirable sex. The primary technique used to produce all male stocks in Nile tilapia has been sex reversal induced by steroid hormones, where synthetic androgens are used as masculinizing agents. Androgens are usually incorporated into the feed (Pandian and Sheela 1995). Oral administration of synthetic androgens such as 17α-methyltestosterone (MT), and 17α-ethynyltestosterone (ET) has produced all male stocks of Nile tilapia and is a technique that has been successfully applied by farmers. This technique, however, demands a high degree of intervention since it is necessary to keep the fish under controlled conditions, receiving the hormone-treated diet, for approximately 25-30 days after first feeding. There have been recent studies showing that non-steroid compounds, such as enzyme inhibitors, administered orally can produce all male stocks of tilapia (Afonso *et al*. 2001).

Another approach for sex reversal, which has been explored successfully in salmonids, is the administration of steroid hormones through immersion treatments (Donaldson and Devlin 1996). In this approach eggs or alevins are submitted to 2-4 hours immersion in a solution containing the desirable sex reversal agent (Figure 2). There are a few studies in tilapia showing that this technique could be effective in producing all males stocks for tilapia culture (Varadaraj and Pandian 1987, Afonso 1992, Appel 1994, Gale *et al*. 1999). Among the advantages of the immersion technique is the minimal intervention, which is usually reduced to the hours when the fish are most sensitive to the treatments.

To effectively induce sex reversal through administration of androgens or non-steroid substances (via oral or immersion techniques), some key points must be considered. First, the substances need to be administered during the period when the gonads are still undifferentiated. In contrast to mammals, sex differentiation in fish usually happens after hatching and varies with the species. In tilapia, at 25-30 days post hatch (dph) it is possible to histologically distinguish ovaries from testis, indicating that gonadal sex differentiation has taken place. Within this window of undifferentiated gonads, from hatch to 25-30 dph, it is important to determine the timing when fish are most sensitive to the treatments. Previous studies have shown that the androgens MT and androstenedione applied at 7 to 14 dph through immersion can skew sex ratio in Nile tilapia (Afonso 1992, Appel 1994).

It is also necessary to determine the best dosage, frequency and duration of the immersion treatments, and the most efficient agent to use. While the dosages tested have ranged from 100-2000 μg/l, frequency and duration vary from a single immersion for 2-4 hours, double immersion at different days for 2-4 hours and continuous immersion for

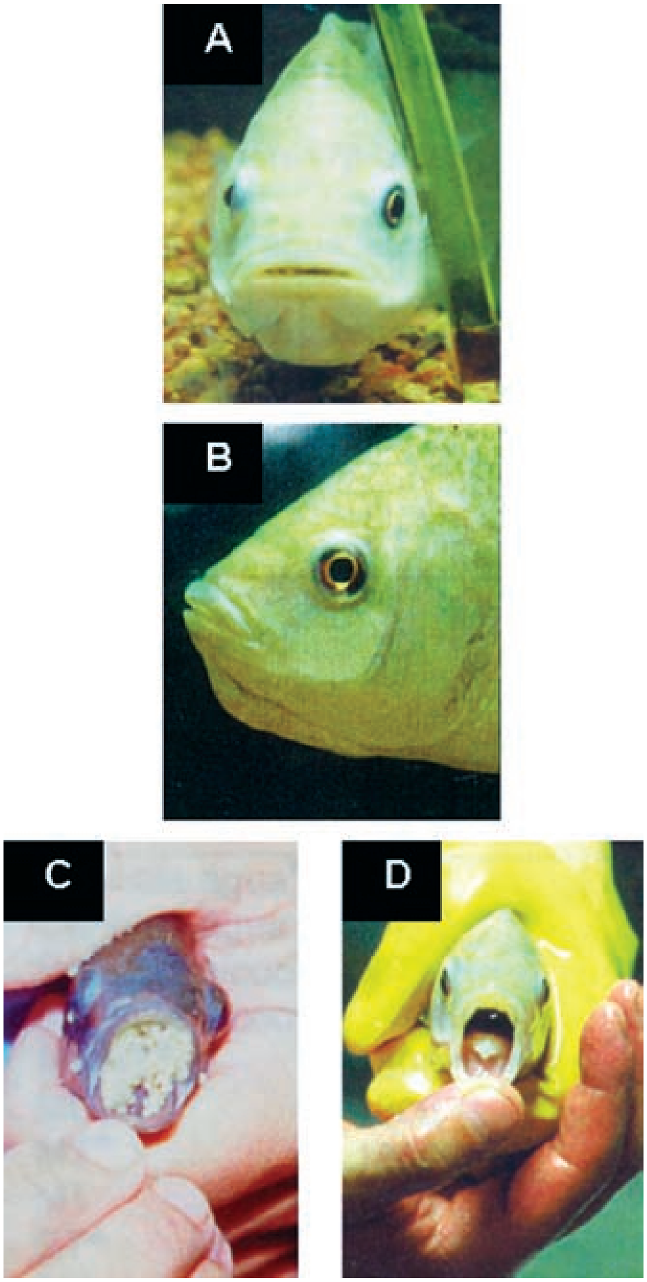

Fig. 1. Female Nile tilapia incubating eggs orally. A-B, show the changes on oral cavity conformation (250 g female); C, eggs inside the mouth (25 g female); D, oral cavity after washing out the eggs.

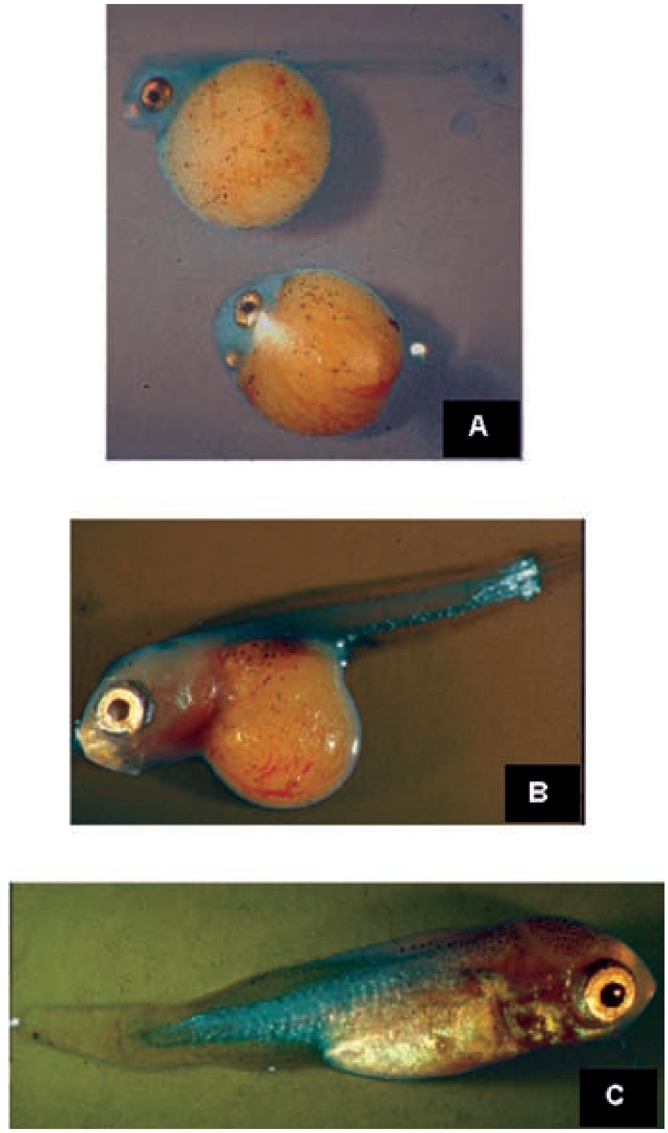

Fig. 2. A, Nile tilapia egg one day before hatching (bottom) and larvae one day after hatching (upper); B, larvae 3-4 days after hatching; C, larvae 7-8 days after hatching.

several days. Among the most common androgens used for masculinization are: MT, ET, and 17α-methyl-dihydrotestosterone (MDHT). MT and ET are aromatizable androgens, which means they can be converted to estrogens by an enzyme called aromatase. This characteristic makes MT and ET sometimes less potent than MDHT, which is not aromatizable. Besides, inasmuch as aromatizable androgens can be converted to estrogens, they may have a feminizing effect, instead of a masculinizing one and this is called paradoxal feminization.

In this study we investigated the masculinizing potency of ET, MT, and MDHT applied to Nile tilapia larvae, at 14 dph, by immersion treatment. In a first trial, 14 dph Nile tilapia larvae were subjected to a single 4 h immersion treatment in ET, MT, and MDHT at concentrations ranging from 200-1800 μg/l. In a second trial, larvae were exposed to the highest concentration (1800 μg/l) of these androgens for 4 h applied either as a single immersion (IM) (1 IM at 14 dph or double immersion (2 IM at 10 and 14 dph). The theoretical sex ratio in most species of fish is 50 percent males and 50 percent females; however, naturally there is a deviation from this proportion. For example, 40:60 for either of the sexes is quite common. Therefore, in sex reversal studies, it is important to have a control group (non-treated) in which the sex ratio can be compared with the treated groups.

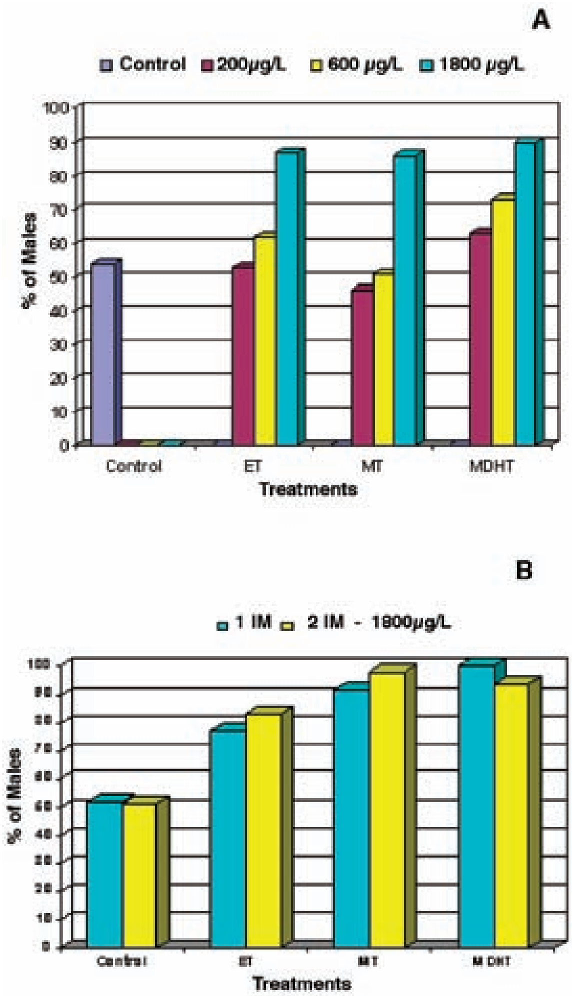

Fig. 1. Masculinization rate in Nile tilapia on the first (A) and second (B) trials.

Immersion Treatments and Masculinization Rates

In the first trial (Figure 3A), the proportion of males in the control group was near 50 percent. In groups treated with the lowest concentrations of MT, ET and MDHT (200 and 600 µg/l) the proportion of males was similar to control group, demonstrating that those treatments did not significantly increase the proportion of males. However, the highest concentration (1800 µg/l) significantly increased the proportion of males in all androgens tested (ET 87 percent; MT 86 percent; MDHT 90 percent).

The results of the second trial (Figure 3B) also showed that the proportion of males in the control groups was near 50 percent. All the three androgens (1800 µg/l) significantly increased male proportion (ET 87 percent; MT 86 5; MDHT 90 percent), confirming what was observed on the first trial. Compared with one immersion, two immersions slightly increased male proportion in ET (1 IM 77 percent; 2 IM 83 percent) and MT-treated (1 IM 92 percent; 2 IM 98 percent) groups, but decreased male proportion in MDHT-treated group (1 IM 100 percent; 2 IM 94 percent).

Practical Considerations

In contrast to the salmon farm industry, which already makes practical use of the immersion technique, this sex reversal technology is still at the laboratory stage for tilapia. Several aspects should be investigated before this technology can be applied at the farm level. We would like to address some of the important findings of this study and of the immersion technique and relate them to a practical approach. Firstly, we showed that through a single 4 hours immersion in a variety of androgens it is possible to significantly skew sex proportion in Nile tilapia and to achieve high rates of masculinization. This confirms previous studies in our laboratory (Afonso 1992, Appel 1994). Secondly, the three androgens tested increased the proportion of males and there was a clear dose-response effect, where the highest concentration tested produced the greatest proportion of males. However, comparison of masculinization rates between trials, highest concentration treatments in trial 1 vs. a single immersion in trail 2, revealed how variable the results can be; the same experimental conditions were used in both trials, except that larvae were from different broods. This inconsistency is one of the drawbacks of this technique. It indicates how much research is required to get consistently high rates of masculinization, which is already possible to achieve when androgens are incorporated in the feed. Thirdly, this study demonstrated that 14 dph is an adequate time to apply immersion treatments, confirming our previous result (Apple 1994). Fourthly, it may be possible that other factors, such as stocking density and temperature during the immersion, which we did not test, are affecting the efficiency of the androgen treatments. In our studies we have used between 120-130 larvae per treatment. Because the treatments were carried out in 2-l glass aquaria, our stocking density was approximately 60 larvae/l or 6600/m^2. Regarding water temperature, our studies have been conducted at 27-30°C.

To apply the immersion treatment at the right time, it is necessary to know when the larvae hatch. As mentioned before, Nile tilapia females incubate eggs orally and they may keep fry in their mouths up to 15 dph. It is therefore essential to have control over the incubation process. In practical terms, it means that the eggs must be removed from the female's mouth and incubated artificially. The increase in labor, space and technology involved with these aspects of artificial incubation may be a negative aspect of this technique. However, around the world there has been an increase in tilapia farmers using the artificial incubation technology. The rationale behind this change derives from studies showing that removal of eggs from the female's mouth reduces the interval between spawns, thus increasing number of larvae produced. The farmers, however, are using the diet as a preferable route to masculinize the larvae. Usually when sex reversal is applied through the dietary route, Nile tilapia larvae are collected from the broodstock tanks, after the females have released them. Starting hormone treatments at this time, approximately 15 dph, it may reduce the efficiency of the sex reversal, since the larvae will start to receive small dosages of the masculinizing agent when they already are in the process of sexual differentiation. Although the artificial incubation process, which needs to be used in the immersion technique, can be more labor intensive, it allows for a better control of all stages of the embryonic and larval development. It may even be useful for genetic selection programs.

Since 1990, our laboratory has been

developing techniques to artificially incubate Nile tilapia eggs. We have also studied the effects of starting to feed androgen-treated diets to larvae as early as 7-8 dph. Our results demonstrate that we could consistently produce all-male stocks not only under laboratory, but also under field conditions. It would be interesting to carry out a study comparing the efficiency of the existing protocols for sex reversal (oral *vs*. immersion). Aspects such as: period of intervention, amount of hormone or another substance used, protocol for setting up the treatments, incorporating hormone into the feed *vs*. preparing the bath treatment, cost-benefit, and human exposure and contact with the masculinizing agents should be investigated.

Other hormones and non-hormone substances capable of influencing sex steroid hormone metabolism should be tested as sex reversal agents in immersion treatments. For example, aromatase inhibitors have been shown to alter sex proportion in several animal species (Afonso *et al*. 2001). Recently we have shown that the aromatase inhibitor Fadrozole, incorporated in the feed can produce high rates of masculinization, 90-100 percent (Afonso *et al*. 2001). It would be worthwhile to explore the use of aromatase inhibitors through immersion treatments. Another aspect that needs further investigation is applying immersion treatments at different stages of development. In chinook salmon, it is possible to achieve complete sex reversal applying a single two hour androgen immersion one day before hatching (Baker *et al*. 1988).

Final Considerations

We have shown that it is possible to increase the proportion of males in Nile tilapia applying androgens (MT, ET, and MDHT) through the immersion technique. Furthermore, 14 dph is a sensitive time for the immersion treatments. Unquestionably, it is far from the point when the androgen immersion technique could be used at a commercial level in tilapia culture. Several variables must be further investigated to maximize and consistently produce high rates of masculinization. When this technique is further tested and improved, it will be of great benefit to the industry, mainly by reducing the period of intervention during sex reversal. It may even be possible to reduce the amount of hormone used.

Notes

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References

Afonso, L.O.B. 1992. Reversao sexual da tilapia nilotica (*Oreochromis niloticus*) atraves de tratamentos de imersao apos a eclosao utilizando 17-metiltestosterona. M.Sc Dissertation. Federal University of Rio Grande do Sul, Porto Alegre, RS, Brazil.

Afonso, L.O.B., G.I. Wassermann and R.T. Oliveira. 2001. Sex reversal in Nile tilapia (*Oreochromis niloticus*) using a nonsteroidal aromatase inhibitor. Journal of Experimental Zoology 290:177-181.

Appel H.B. 1994. Masculinizacao de pos-larvas de tilapia do nilo (*Oreochromis niloticus*) utilizando androgenos atraves de tratamentos de imersao. M.Sc Dissertation. Federal University of Rio Grande do Sul, Porto Alegre, RS, Brazil.

Baker, I.J., I.I. Solar and E.M. Donaldson. 1988. Masculinization of Chinook salmon (Oncorhynchus tshawytscha) by immersion treatments using 17-methyltestosterone around the time of hatching. Aquaculture 72:359-367.

Donaldson E.M. and R.H. Devlin. 1996. Uses of biotechnology to enhance production. Pages 969-1020 In W. Pennel and B. Barton, editors. Principles of salmonids culture. Elsevier, Netherlands.

Gale W.L., M.S. Fitzpatrick, M. Lucero, W.M. Contreras-sanches and C.B. Schreck. 1999. Masculinization of Nile tilapia (*Oreochromis niloticus*) by immersion in androgens. Aquaculture 178:349-357.

Pandian T.J. and S.G. Sheela. 1995. Hormonal induction of sex reversal in fish. Aquaculture 138: 1-22.

Varadaraj, K. T. and J. Pandian. 1987. Masculinization of *Oreochromis mossambicus* by administration of 17a-methyl-5-androsten-3b-diol through rearing water. Current Science 56:412-413.