EFFECT OF FISH DENSITY ON EFFICACY OF MASCULINIZATION BY IMMERSION IN MDHT

Eighth Work Plan, Reproduction Control Research 2B (RCR2B)
Final Report

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ABSTRACT

The effect of fish density on the capacity of the synthetic androgen 17α-methyldihydrotestosterone (MDHT) was investigated. As in previous studies in this laboratory, significant masculinization occurred when fish were immersed in 500 µg l^{-1} of MDHT for two hours at 280 and 364 CTU at a density of 33 fish l^{-1} (80.3% males vs. 56.7% males in the controls). When the density during treatment was increased to either 66 or 100 fish l^{-1}, MDHT immersion resulted in 71.7% males in both treatments, which was nearly significantly more than controls, and suggests an effect of stocking density on masculinization.

INTRODUCTION

Masculinization of tilapia for the production of male-biased populations continues to be an important tool for aquaculturists to prevent unwanted reproduction (which shunts energy away from growth towards gamete production) and to produce the sex with the larger growth potential. Previous work in our laboratory has shown that short-term immersion in androgenic steroids can result in masculinization of Nile tilapia (Gale et al., 1995; Fitzpatrick et al., 1998). These studies show that immersion in androgen has the potential to be an alternative to dietary treatment with steroids for the masculinization of tilapia. A variety of androgens—especially synthetic androgens—are effective masculinizing agents (Hunter and Donaldson 1983); however, there may be differences in their potency. Fitzpatrick et al. (1998) showed that a single immersion in the non-aromatizable synthetic androgen 17α-methyldihydrotestosterone (MDHT) at 364 CTU (Day 13 post-fertilization at 28°C) was as effective as two immersions in MDHT at 280 and 364 CTU. Either treatment resulted in significantly more males being produced than in the controls. While these results demonstrated the potential for immersion, they did not determine optimal treatment conditions. Another androgen trenbolone acetate (TBA) was recently reported to be an effective masculinizing agent when fed to tilapia (Galvez et al., 1996). While we have had variable success masculinizing tilapia by immersion in 17α-methyltestosterone (MT), we have recently begun to examine the efficacy of immersion in TBA.

In order to determine the best treatment conditions for masculinization by immersion, studies must be conducted on those factors that are believed to play a critical role in determining efficacy. For masculinization by immersion, the major factors are type of hormone, timing of treatment (relative to fish development), hormone dosage, duration of exposure, and density of fish during immersion. In both of these studies, the density of fish during immersion was maintained at 33 fish l^{-1}. Fitzpatrick et al. (1998) showed that significant masculinization by single immersion in MDHT could only be achieved by exposure at 364 CTU (Day 13). No significant masculinization effects were observed in groups immersed once in MDHT at either 280 (Day 10) or 310 CTU (Day 11). Thus, we have narrowed the period during which Nile tilapia are sensitive to immersion to after Day 11 in MDHT at 500 µg l^{-1}, at 364 CTU, and at 33 fish l^{-1}.

Because density, hormone dosage, and length of exposure are factors that may interact, a factorial design is needed to know the minimum dosage required, the highest density to use, and the shortest exposure needed. Little information has been generated regarding these factors, and because of a limitation in the availability of fry of a known age, the traditional designs involve one factor at a time. However, when another factor is tested, the best set for the previous factor tested may be affected by interactions among factors.

Because many factors may influence treatment efficacy, conducting a single experiment in which all factors are examined simultaneously at different levels would require large numbers of tanks and more tilapia fry than can be produced from a single spawning. Therefore, our approach up to this time has been to examine one factor at a time while holding all others constant. We will describe such a study on the effects of fish density which was based on the best treatment conditions reported in the PD/A CRSP Fifteenth Annual Technical Report (Fitzpatrick et al., 1998). However, this approach limits the amount of information that can be gained on the interactions among the various factors. Therefore, we will also describe another experiment that was carried out using a fractional factorial design (Kuehl, 1994) to examine multiple factors (hormone dosage, exposure duration, and fish density) simultaneously with the TBA. This design allows information to be obtained on factors of interest in the early stages of experimentation when the number of treatments exceeds the resources (Kuehl, 1994).

METHODS AND MATERIALS

Breeding families of Nile tilapia (*Oreochromis niloticus*) were placed in 200-l aquaria (one male to three females). The temperature was maintained at 28 ± 1°C. Time of spawning was monitored every 2 hours. All spawning occurred between 1600 and 1900 h. Once breeding occurred, the other fish were removed and the brooding female was left to incubate the progeny. At 280 Celsius Temperature Units (CTU) post-fertilization, fry were removed from the tank and randomly assigned to experimental groups. The first experiment was conducted simultaneously with the experiment reported in Fitzpatrick et al. (1998). Double immersions spaced several days apart (see below) were chosen as the best treatment timing. The second experiment used double immersions on consecutive days (see below) as the best treatment. Development of the fry was expressed in CTUs (mean water temperature in °C x the number of days since fertilization). The fry used in the experiment came from an individual female. Each replicate was housed in a 3.8-l glass jar with dechlorinated tap water. The water in all treatments was maintained at 28 ± 1°C under constant aeration. Treatments consisted of immersions in either steroid or ethanol (EtOH), which were mixed before addition of fry. Steroids were obtained from Sigma Chemical Company (St. Louis, Missouri) and stored in stock solutions of ethanol (1 mg ml^{-1}).

For experiment 1, fry were immersed for two hours at 280 and 364 CTU (10 and 13 days postfertilization; dpf), in 500 µg l^{-1} of MDHT at densities of 33, 66, 100, or 200 fish l^{-1} in each replicate. Fish in the EtOH control group were immersed at 280 and 364 CTU at a density of 33 fish l^{-1}. Each experimental group was triplicated.

For experiment 2, a fractional factorial design was used to examine the effects of fish density, hormone dosage, and exposure duration simultaneously. Fry were immersed at 364 and 392 CTU (13 and 14 dpf) in either TBA or EtOH. Fry densities were 12, 25, 50, 100, or 200 fish l^{-1}; hormone dosages were 62.5, 125, 250, 500, or 1000 µg l^{-1}; exposure duration was 0.75, 1.5, 3, 6, or 12 h. Because a fractional factorial design was used, only certain combinations of treatment conditions were used. To choose the combination of treatment factors to be used, a model was generated using Statistical Analysis Systems for Windows. Under this model, only replication around the middle treatment level for each factor is recommended. The fractional factorial design is effective in screening studies to check on many factors, with the assumption that only a few effects are important. However, the fractional factorial design carries the caveat that follow-up experiments must be conducted using suitable replication once the levels for the various factors are chosen.

Fry were collected after each immersion, jars were thoroughly cleaned, and then fish were reallocated in fresh dechlorinated tap water. Seven days after the final immersion, fry were transferred to Oregon State University's Warm Water Research Laboratory, Corvallis, Oregon, and reared in 75-l fiberglass tanks in a recirculating system. Temperature and pH were monitored daily; ammonia, nitrites, dissolved oxygen, alkalinity, and hardness were measured weekly. Water temperature in the grow-out system was maintained at 28 ± 1°C. At 60 to 70 dpf, sex ratios were determined by examination of gonads using squash (10 and 40X) preparations after aceto-iron hematoxylin staining (Wittman, 1962). The weights of sampled fish were recorded at this time.

For experiment 1, data were pooled from replicate tanks because there was no evidence of tank effects within treatments (Fisher's test or ANOVA). Sex ratio and mortality data were analyzed using Fisher's exact test with exact p-values (a more conservative test than the chi-square test for small sample sizes) estimated in GraphPad Prism™. The mean final weights of sampled fish were analyzed for differences between groups using one-way ANOVA, including mortality as a possible confounding variable. For all analyses, differences were considered statistically significant when the p-value (P) was less than 0.05. For experiment 2, the data were not analyzed statistically because of the sex ratio bias (see Results). Had the results been unbiased, the data obtained for each treatment combination would have allowed for the creation of a response surface. The response surface, contours primarily formed by linear and quadratic equations, may show how the response increases or decreases based on the interaction of the factors tested and indicates trends along levels of the factors.

RESULTS

Experiment 1: Effects of Density

Treatment with MDHT resulted in masculinization of tilapia (Figure 1). The percentage of males in the 33 fish l^{-1} treatment was significantly higher than in the control group (80.3% vs. 56.7%; $P = 0.004$), whereas the percentage of males in the 67 (71.7%) and 100 (71.7%) fish l^{-1} treatments were not quite significantly different from controls ($P = 0.06$). The proportion of males in the only replicate of the treatment with 200 fish l^{-1} (64.5%) was not significantly different from the control.

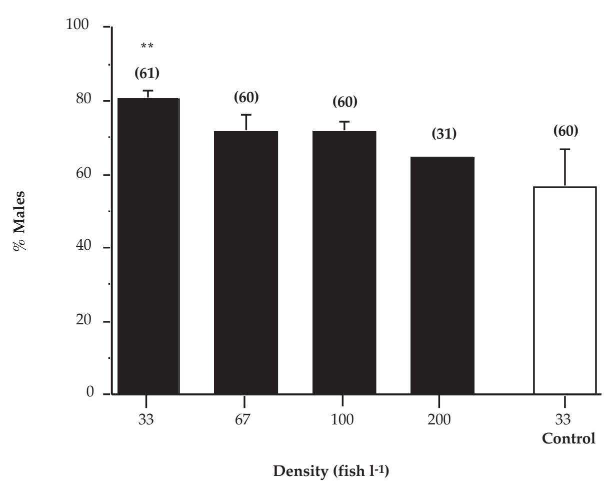

Figure 1. Effect of different fish densities during treatment on masculinization of Nile tilapia (*Oreochromis niloticus*) fry by two MDHT immersions. Sample size for each density is shown in parentheses. Masculinization ratios that were significantly different from controls are represented by asterisks ($P < 0.01$).

Mortality and final weight data were not significantly different among treatment groups. Water quality in rearing tanks was maintained close to the optimal values for tilapia culture (data not shown).

Experiment 2: Fractional Factorial

All control groups (immersed in EtOH or unimmersed) and treatment groups contained 100% males. Therefore, the data were not analyzed further.

DISCUSSION

Significant masculinization occurred when fish were immersed in 500 µg l^{-1} of MDHT for two hours at 280 and 364 CTU at a density of 33 fish l^{-1}. The ratios of males produced by MDHT immersion at the 66 and 100 fish l^{-1} were nearly significantly different than controls, and suggest an effect of stocking density on masculinization. The most effective stocking density in this study was 33 fish l^{-1}, which is nearly five times that reported by Torrans et al. (1988) in a study in which *O. aureus* were masculinized by immersion for five weeks in mibolerone.

Although a stocking density can be recommended on the basis of this experiment, further study is needed to assess the interaction of the major factors that influence susceptibility to androgen-induced masculinization. The fractional factorial design presented an opportunity to explore the influence of several major factors and their interactions; however, the control fish turned out to be all male. Such extreme sex ratios in tilapia, while obviously problematic in the current study, are not unusual (see Shelton et al., 1983). Nevertheless, determination of the effects of TBA dosage, fish density, and treatment duration on masculinization must be conducted using a brood with a more balanced sex ratio. An experiment using a similar fractional factorial design is now underway in our laboratory.

ANTICIPATED BENEFITS

We have successfully refined the technique for masculinizing Nile tilapia by immersion in masculinizing steroid. This latest development defines one of the key variables that affects the success of immersion and provides information critical to the use of immersion as an alternative to dietary treatment with androgens for sex inversion. The development of this new technology for masculinization of tilapia may enable farmers to masculinize tilapia with androgens while minimizing the risk of pond contamination with MT. Defining the critical stage of development when tilapia are susceptible to masculinization by immersion may also provide important clues as to when tilapia are susceptible to masculinization by treatments that do not involve hormones (e.g., temperature and pH).

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