Global Journal of Fisheries and Aquaculture

ISSN: 2408-5464 Vol. 2 (5), pp. 177-181, October, 2014. © Global Science Research Journals
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Full Length Research Paper

Comparison between greenwater and clearwater hatchery systems during the masculinization process of silver tilapia, *Oreochromis noloticus* (Perciformes: Cichlidae)

Running heading: Comparison between greenwater and clearwater hatchery systems during the masculinization process of silver tilapia

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Accepted 18 October, 2014

Abstract

The growth and survivorship of Silver Nile Tilapia (*Oreochromis niloticus*) was compared between greenwater systems (containing green algae) and clearwater systems (no algae) while feeding the androgen 17 alpha methyl testosterone. Water chemistry was monitored daily. Algal counts and species identification were done to estimate the composition and density of green algae. Fish length, wet mass, mortality and percentage masculinization were calculated. Compared to clearwater, fish raised in greenwater were significantly larger and heavier ($F_{1, 575}$ = 1028.28: p<0.001 for length and $F_{1, 575}$ = 566.48 for mass). In addition there was a significantly higher survivorship in the greenwater system (mean of 85.0% survival in greenwater and mean survival of 61.0% in clear water respectfully, χ^2 =67.18, p< 0.001). There were also significantly higher levels of nitrite and ammonia in the Clearwater tanks. Green water systems were found to be overall superior for producing masculinized male silver tilapia fingerlings, than clearwater systems although no significant difference was noted in percentage masculinized (98.8% green water, 97.7% clear water).

Key words: *Oreochromis niloticus*, masculinization, greenwater system, 17 α methyltestosterone

INTRODUCTION

In Trinidad up to 1997 tilapia production (*Oreochromis mossambicus* and the red hybrid) was greater than 20 tonnes per year, (Ramnarine et al 1998). This number drastically plummeted to less than 1 ton per year until 2008, with a major limiting factor being the availability of male fingerlings for production (personal communication: Mr. C. Barrath, Secretary Aquaculture Association of Trinidad and Tobago Multi-purpose Co-operative Society Limited, Mr. H. Lalla, Fisheries Officer, Ministry of Food Production).

Expected survivorship for all male culture is 90% or greater and by stocking all males, production could be 5.5 tonnes per hectare. (Rakocy et al 1995).

The early investigations into sex reversal of tilapia for fingerling production using hormone 17 α methyltestosterone (Sigma-Aldrich Co. LLC) (MT) treated feed were conducted in aquaria and troughs receiving clear water (Clemens et al 1968; Guerrero et al 1975, Tayamen et al 1978). Indoor tanks have proven to be

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less suitable in comparison to outdoor tanks and had greater mortality (Phelps et al 2000). Popma (1987) reported 40% survival with *O. niloticus* when sex reversed at a stocking density of 3200 to 4500 fry per m^3 in indoor tanks. Vera Cruz et al (1994) had obtained no more than 70 % survival utilizing outdoor tanks stocked at 5000 per $metre^3$ though. Of particular importance is Bundle's (1984) research, in which a comparison between the use of indoor tanks and clear water outdoor tanks and hapas in static water ponds was made during the sex reversal process for tilapia. Here 96.0 to 98.0 % males were obtained from those held in the hapas and those treated in indoor or outdoor tanks. Chambers (1984) also received a high percentage of males working with *O. niloticus* in hapas in fertilized earthen ponds or fertilized static water in outdoor tanks. Phelps et al (2000) reported Stoking fry at 3000 to 4000 per m^3 in hapas or flowing water tank ensured an active feeding response needed so all fish can consume feed.

Keeping in mind that the genus *Oreochromis* is microherbivorous (Barlow 2000), it is hypothesized that *O. niloticus* fry grown in a greenwater (GW) system will have greater increases in mass and length, than those grown in a clearwater (CW) system during the masculinization process. Secondly we hypothesized there will be less fry mortality in a GW system. It is also believed that the percentage of maculinization of fish will not be affected in the GW aquaria system therefore it is aimed at indicating advantages of GW hatchery system over a CW hatchery system.

METHOD

Eight tanks measuring 45.72cm x 20.32cm x 20.32cm were used. Four of these tanks were used as the green water (GW) tanks and four with clear water (CW). All tanks were filled with water to a depth of 15cm. Water for both treatments were de-chlorinated by using approximately 0.2 g of sodium thiosulphate per tank. All eight tanks were aerated equally by using a gang valve to regulate the air pressure and volume. Black plastic bags were used to block sunlight penetration in CW tanks. These tanks were also covered with styrofoam sheets. The GW treatment tanks were seeded with 100 ml of water from an intensive green water tank housing brood stock *Oreochromis niloticus*

O. niloticus fry were collected from breeding tanks at the fish farm. A hand net was passed along the surface, near the edge of the tank, (at approximately 8:15am), as suggested by Popma et al (2000) to obtain day old fry. Each tank was stocked with exactly one hundred fry. Fifty fry were randomly selected before stocking, to be weighed and measured. The temperature of the water in all eight tanks was monitored daily at the same time every morning (0830 hrs). All tanks were cleaned daily by removing1/3 of the volume water from the base of the tanks with 1.0 cm diameter siphon. The CW tanks were all refilled stored clear water and the GW tanks were all refilled with water from an adjacent green water tank that was seeded with water from the original breeding tank.
Water samples were taken on the Day 7, 14, 21, and 28 during the hormonal treatment. Ammonia, pH, nitrite and algal density were monitored. The algal density was estimated using a Sedgewick-Rafter Counting Cell and a low power light microscope. The pH was tested with a pH pen (Eutech Instruments) and ammonia and nitrites were tested using the relative reagents and apparatus from the La Motte's freshwater aquaculture test kit (MD, USA). The GW samples were filtered with a Buchner funnel and Buchner flask before the water test was carried out. Daily record of mortality was kept for each tank.

The feed was prepared by using ground pellets that had a 40% protein composition. Sixty mg of 17 α methyltestosterone (MT) was dissolved in 100 ml 95% ethanol. The solution was combined with 1.0 kg of ground feed. The mixture was then allowed to air dry and shielded from sunlight to prevent protein disintegration by ultra-violet light. For the first 14 days of feeding the fish in all tanks were fed '*ad libitum*' (i.e. in excess). This was done four times per day.

On the Day 14 a sample of ten fish from each tank was taken to obtain their mass and length. The fish were anesthetized using 0.5g of ethyl 4 aminobenzoate to 0.3g acetone and diluted with 4.0L of water. The fish were immersed in anesthetic for about one minute, then taken out swabbed on tissue paper, weighed and measured. Following this the fish were revived in heavily aerated water (Popma et al 2000).

From the Day 15 the fish were fed 20% of their total body mass daily (divided into four feeding sessions) Phelps et al (2000). On day 28 the hormonal diet treatment was concluded. On Day 29 the surviving fish were counted, weighed and measured while anesthetized again.

A General Linear Model (GLM) was applied to test whether differences in standard length (SL) and wet weight (wt) of fish were explained by green and clear water treatments. The water treatment (GW and CW) was a fixed factor since the alternative hypothesis expects directional effects, i.e. higher growth rates, weight and standard lengths in the GW treatments. The tank number was a random factor, since no effects were expected between replicated tanks. Each treatment was replicated four times, and algal counts were made during the experiment's 28 days and the means were used for the following calculations.

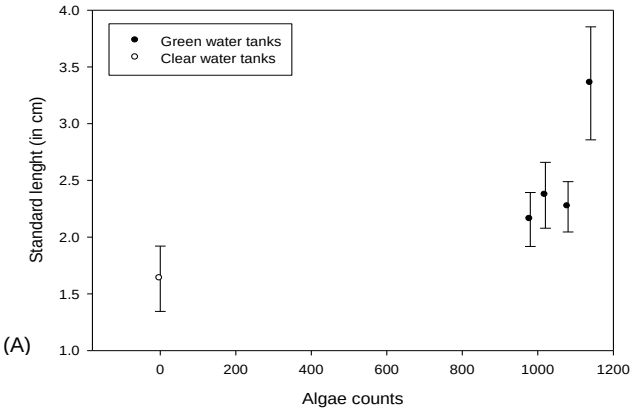
There were substantial (>10%) differences in algal counts in the GW treatments and there were significant variation between the replicates (i.e. the GW tanks). To account for this unexpected variation, an additional test was performed. Besides the planned test, tanks were replicated and a random factor, the data were also analyzed with algal counts as co-variants using a model II regression analysis.

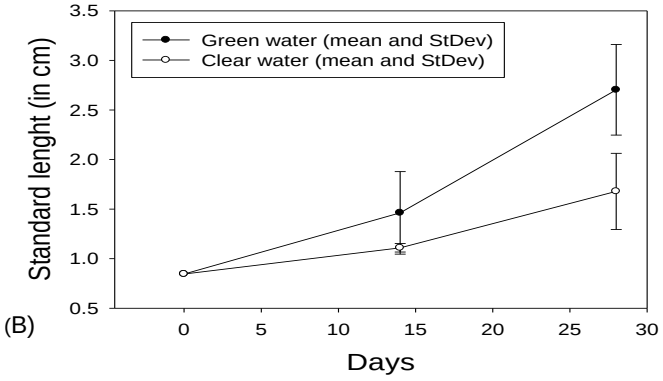
Table 1: GLM of Log (SL) with Treatment as fixed factor and Tanks as random factor.

Source	d.f. (Degree of freedom).	Ms (Mean of Square)	F	p
Treatment	1	25.2613	1028.28	0.000
Tanks	3	2.5984	105.77	0.000
Error	575	0.0246		
Total	579			

Table 2: GLM of Log (Wt) with Treatment as fixed factor and Tanks as random factor.

Source	d.f. (Degrees of freedom.)	Ms (Mean of Square)	F	p
Treatment	1	150.981	566.48	0.000
Tanks	3	17.500	65.66	0.000
Error	575	0.267		
Total	579			





Prior to performing the GLM, all data were natural-log transformed and the assumptions of parametric analysis were tested, i.e. normality of residuals (using a Ryan-Joiner test) and homogeneity of variances (using a Bartlett test), (Sokal et al 1995). All data analysis was performed in Minitab 12.1.

All surviving fish were housed in uniform conditions in concrete enclosures maintaining their treatment integrity only and were examined approximately 2 months after the trial to confirm their sex.

RESULTS

Assumption Tests

Normality test (R = 0.9972, p = 0.0395), shows that the natural logarithmic transformed Standard Length (SL) data are approximately normally distributed. In addition, the variances of SL are not homogeneous, and in particular, the clear water treatments show the highest variance (Bartlett's test statistic = 64.40, $p < 0.001$). This indicates that the results should be interpreted with caution, and the critical value for tests were made more conservative and reduced from $\alpha = 0.05$ to $\alpha = 0.01$ to reduce a type I error (erroneously observing a significant differences where there is none, (Sokal et al 1995). Qualitatively similar results were obtained from analyses on natural log transformed weight data (Normality test, R = 0.9853, $p < 0.001$, and Bartlett's test for homogeneity of variances = 64.63, $p < 0.001$).

Data analysis- Standard length and weight

Table 1 shows there were highly significant ($F_{1, 575}$ = 1028.28, $p<0.001$) differences between treatments in that the mean (±SE) of the GW treatment fish were higher that those of the CW treatment, (1.633(±0.018) and 2.516(±0.0311), for the GW and CW respectively). Table 1 also shows there were significant differences between tanks within treatments, suggesting that the variation in algae counts between tanks may have affected the growth of fish.

Table 2 shows that the variation in weight of fish was also affected by both treatments and tanks.

In order to account for this unexpected variation between replicate tanks, an additional test was performed in which the algal count was used as covariant, applying a model II regression analysis. This analysis shows that the algal density explained significant variation in SL of fish between tanks (T = 28.49, $p < 0.000$, R^2 = 58.4%), see Figure 1. Similarly, the weight of fish was also significantly affected by algae densities (T = 23.08, $p < 0.000$, R^2 = 48.0%).

Figure 1. Clearwater treatments have no algae and the mean and standard error are therefore not separately calculated for these tanks (A). Standard length (mean and standard deviation (B)) of fish after 28 days in the clear water (open symbol) and greenwater treatments (solid symbol) in tanks with variable algal counts.

Survival Analysis

Variation between tanks in survival of fish were analyzed using a Chi-squared test, which showed that the survival differed significantly between tanks (χ^2 = 80.66, d.f.=7, p < 0.001). In addition, clear water tanks showed the lowest survival (61.0%), significantly lower (χ^2 = 67.18, d.f.=1, p < 0.001) than the green water tanks (86.5%).

No statistical difference in number of males were detected between both treatments two months after MT feed treatment concluded (98.8% greenwater, 97.7% clear water)

Water Quality Analysis

Greater fluctuations of nitrites and ammonia were noted in the CW systems but no significant variations among them. Lower levels of both nitrites and ammonia were noted in the GW systems (χ^2 = 63.28, d.f.=1, p < 0.001) in comparison to the CW. There was no variation of temperature and pH between both treatments.

DISCUSSION

This study shows that decreased mortality and increased weight gain were statistically significant for greenwater (GW) treatments in comparison to clearwater (CW) systems for fingerling production during the sex reversal (masculinization) process. This can be seen from the statistical analysis using the General Linear Model (GLM). This model was chosen because it is a parametric test and the data fulfills the requirements for such a test. This test also provided the opportunity to incorporate varying algal counts into the calculations. The observations were not normally distributed and the variances were not equal. This was the main reason that Natural Log values (log e) were used, since it provided a means of normalizing the data for the distribution of the GLM ANOVA and unequal sample size. Table 1 and Table 2 showed the high F values of the treatments. This also showed there is a significant difference between CW and GW treatments with regards to weight and length of fish on Day 29. A simple Chi square test was chosen to compare the mortality or survivorship in green and clear water tanks. This was chosen to simplify the ANOVA test with another parameter being measured. This test showed there was conclusive evidence to also prove that GW tanks have better survivorship than CW tanks.

Both the ammonia and nitrite levels were high in the CW tanks but remained fairly low and constant in the GW tanks. The difference between green and clear water system can be attributed to the photosynthetic action of the green algae that utilized nitrates. Additionally the high difference in ammonia levels contributed to higher mortality in the CW tanks. Over the duration of the experiment there was a continuous increase in chemical concentrations, although water changes were conducted. Unlike clearwater, algae continuously removed the nitrates resulting in a decrease in concentration. This provided the opportunity for bacterial activity to further convert the ammonia and nitrites to nitrates. Even though the nitrite levels rose to approximately 1.0 gl^{-1} and the ammonia rose to approximately 3.0 gl^{-1}, the water quality did not deteriorate to concentrations harmful to *O. niloticus*. This was supported by Ardjosoediro and Ramnarine (2003) who reported tilapia surviving ammonia concentrations of 2.3gl^{-1}.

Mortality during the first week was due to the stress on the day old fry from stocking. The results indicate that the increase in mass and length of fish in green water was due to the high density of green algae. The green algae in tanks 1, 2, 3 and 4, supplemented the diet of the fish. *O. niloticus* is micro-herbivorous so in conjunction with the sex reversal hormonal (MT) treated feed, there was both an increase in mass and length. The CW fish had no such supplements. Also the algae were responsible for the reduction of the ammonia and nitrites. They removed nitrates during growth. This increase in algal population contributed to further increase in growth as seen in tank 1. The growth rate of fish increased to approximately 0.9 cm $week^{-1}$ whilst in tank 2, 3 and 4 it was approximately 0.7cm $week^{-1}$. The increased density of algae in tank 1 was attributed to it receiving an additional hour more of direct sunlight daily. This resulted in increased photosynthesis and growth. Marjani et al (2009) indicated a dose rate of 75 mg kg^{-1} MT of feed resulted in maximum male population (98.09%) and gave the maximum gain in body weight in comparison to 50 mg kg^{-1} and 100 mg kg^{-1}.

The high mortality of tank 1 as compared to tank 2, 3 and 4 was most likely because of increased competition for food and space and territorial behavior.

All fish from the experiment were grown to three months old and were manually sexed and determined to be all males. It can be concluded that green water hatchery system has no effect on the potency of the masculinization hormone, 17 α methyltestosterone, and enhances the production of male fingerling production in a hatchery.

Between 2008 to 2012 there has been an increase of Tilapia production to approximately 5 to 10 tonnes per annum (Fisheries Division, 2012). This can be attributed to hatchery facilities by both state and privately owned. Off these at least one privately owned hatchery (author's observation) has employed greenwater systems for 'super male'(Mair et al 1997) breeding stocks and fry production and is having favorable production results.

ACKNOWLEDGEMENTS

We would like to thank Dr. C. Van Oosterhout of University of East Anglia, UK, for assisting with the

statistical analysis of data and the Department of Life Sciences, UWI, for funding this project.

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