Reproductive Efficiency, Fry Growth, and Response to Sex Reversal of Nile and Red Tilapia

Interim Workplan, Africa Study 6

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Introduction

The red color pattern in some strains of tilapia has added commercial value in some markets. Although red tilapia may be more marketable, questions have arisen regarding its value as a culture fish. El Gamal (1987) reported that during growout from 130 to 262 g, a red strain of tilapia had significantly lower survival than *Oreochromis aureus x Oreochromis aureus* and *Oreochromis niloticus x Oreochromis niloticus. O. niloticus* have shown superior growth during the sex-reversal phase compared to red tilapia (Berger and Rothbard, 1987). Red females produced the same number of fry per kg of body weight as *O. niloticus* females (El Gamal, 1987).

In the following study, *Oreochromis niloticus* and a red x red (RT x RT) strain were compared in terms of fecundity of brood females and efficacy of sex reversal.

Materials and Methods

Research for these studies was conducted in two phases at the El Carao National Fish Culture Research Center, General Directorate of Fisheries and Aquaculture, Ministry of Natural Resources, Comayagua, Honduras.

Phase I-Broodstock Fecundity

Red tilapia broodstock were identified electrophoretically as 80% *O. niloticus,* 12% *O. aureus*, and 8% *O. mossambicus.* All 11 loci examined were contaminated to some degree. Analyses were conducted by Fishery Information Management Systems, Auburn, Alabama, using starch-gel electrophoresis (McMandrew and Majumdar, 1983; Macaranas et al., 1986; Brummett, 1986). Only red broodstock were used for reproduction trials. Red brooders were a light, pinkish-red color with an occasional black spot near the operculum.

Nile tilapia (*O. niloticus*) from Auburn University were introduced to El Carao Station in 1977. In 1988 they were identified using horizontal starch-gel electrophoretic techniques (Abdelhamid, 1988), as 90% *O. niloticus* with four contaminated loci:

- GPI-A from *O. mossambicus*,
- ACP-B from *O. mossambicus* or *O. hornorum*,
- SOD-A from *O. mossambicus* or *O. hornorum*, and
- MDH-A from *O. mossambicus* or *O. hornorum* or *O. aureus*.

Seed Production

Four 0.05-ha ponds were filled with reservoir water two to three days prior to stocking. Two ponds were stocked with red broodfish and two ponds with Nile tilapia broodfish per trial. Prior to stocking, a sample of 25 males and 25 females from each group were weighed and measured to calculate Fulton's Condition Factor (Anderson and Gutreuter, 1983). Two hundred and thirty females and 115 males were stocked into each pond (882 -1,554 kg/ha). The mouth of each female was checked for eggs or fry at stocking. Broodfish were fed a 25% protein ration once daily at a rate of 1% body weight per day. Maximum and minimum water temperature and morning dissolved oxygen were recorded daily; secchi disk visibility was recorded weekly.

Fry were collected 215 to 230 degree-days after stocking (13-19 days) according to Green and Teichert-Coddington (1993) by draining ponds into a concrete harvest sump in which water was 30-cm deep. The fry were skimmed off the water surface with a large, square-framed, fine-mesh net. Fry were graded through a 3.2-mm vexar screen (Hiott and Phelps, 1993). Fry were counted and sorted into two separate groups: those retained by the grader (> 14 mm) and those that passed through the grader (≤ 14 mm).

Broodfish were collected with hand nets, separated by sex, counted, and weighed. Brooders were held by sex in 20-m^3 concrete tanks and fed at 1.5% body weight per day for seven to ten days prior to restocking. Each spawning pond was thoroughly dried and prepared for refilling. The trial described was repeated four times during a six month period.

Fry were counted by visual comparison to a standard of 500 fry. Subsamples of 25 to 50 fry were measured to the nearest millimeter at the beginning, middle, and end of counting. Direct counts of individuals were taken to determine mortality of fry after harvesting.

A 2 x 4 factorial design, consisting of two treatments and four trials over time, was used; trials were replicated twice per fish type. Treatment means for average weight, number of fry produced, number of fry per kilogram, number of fry per female, length, growth rate, and survival were compared using two-way analysis of variance (ANOVA). Correlations between number of fry and male:female weight ratio, male:female sex ratio, female weight, male weight, condition factor, and temperature were analysed by regression. All statistical analyses were made using a Statview + Graphics program (Feldman et al., 1988).

Phase II: Sex Reversal

Nile tilapia and red fry, 10.4 and 9.4 mm (standard length), respectively, were harvested from Phase I spawning ponds, stocked into hapas suspended from a wooden pier in a 0.1-ha pond with a maximum depth of 1.2 m and a minimum depth of 0.7 m. The hapas measured 1.0 m x 1.0 m x 0.7 m (length x width x height) and contained 0.5 m^3 of water. Fry were counted by visual comparison and stocked at 4,000/m^3 as recommended by Vera Cruz and Mair (1994). When the red fry were stocked, the percentages of red- and wild-colored (black) fry were recorded.

A diet containing 60 mg of 17a-methyltestosterone (MT)/kg of feed recommended by Mair and Little (1991) was prepared according to Zeigler, Inc. methods (personal communication, 1995). The hormone additive was prepared by dissolving 3 g of the steroid in 1,000 ml of 95% ethyl alcohol to make a stock solution of 3 mg MT/ml. The stock solution was stored in a refrigerator at 4°C. Twenty milliliters of the stock solution were added to 210 ml of 90% ethyl alcohol and then sprayed over each kilogram of feed. The feed Zeigler Mash contains 55% protein, 13% carbohydrate, 15% fat, 8-10% moisture content, 8-9% ash, and < 1% fiber. The feed was sprayed in a covered mixer and thoroughly mixed for 20 minutes. Three kg of feed were prepared per batch. The feed was spread in a 5-cm deep layer on a table inside the laboratory at 26°C for 12 hours to allow the solvent to evaporate . The next day the feed was sealed hermetically in plastic zip-lock bags and placed in a freezer at -2°C.

Table 1. Body weight and standing crop of red and Nile tilapia brooders stocked in 0.05-ha ponds from February to July. Data are means of two replicates per trial.

	Avg. Wt. (g)		Standing Crop (kg/ha)	% Survival at Harvest	No. of Fry Harvested	Condition Factor
	Females	*Males*				
TRIAL 1						
Red	136	238	1,092	99.9	33,548	1.6
O. niloticus	120	234	1,024	99.9	36,704	1.6
TRIAL 2						
Red	122	240	1,027	99.9	58,000	1.6
O. niloticus	106	230	976	99.9	55,250	1.6
TRIAL 3						
Red	133	291	1,243	99.9	107,750	1.6
O. niloticus	111	224	1,011	99.9	75,900	1.6
TRIAL 4						
Red	174	338	1,548	99.9	136,000	1.6
O. niloticus	104	266	1,073	99.9	93,500	1.6
GRAND MEAN						
Red	141 *	277 *	1,228	99.9	83,825	1.6
O. niloticus	110 *	238 *	1,021	99.9	65,339	1.6

* Values in the same column are significantly different ($P < 0.05$).

Daily feed quantities were removed from storage, weighed the morning of feeding, and placed in sealed, clear, plastic jars. The jars were held outdoors in white plastic containers next to their designated hapas on the pier of the pond until use. Maximum and minimum water temperature and morning dissolved oxygen (0600) were recorded daily and secchi disk visibility was recorded weekly. Fry were fed four times daily (0800, 1000, 1300, 1600 h) for 14, 21, or 28 days. Feed was placed in 60-cm feeding rings with trays suspended underneath. The feeding rate was adjusted weekly by measuring 25 fish per hapa to the nearest millimeter, and biomass was estimated using the formula described by Shelton et al. (1978). Daily feeding rates were 15, 12, 8 and 4% body weight during weeks 1, 2, 3 and 4, respectively.

After 14, 21, or 28 days of hormone treatment, fry were harvested, weighed, and counted. One hundred individuals from each replicate were randomly selected and measured to the nearest mm to determine length-frequency distribution.

Five hundred fry from each replicate were returned to the hapa and grown to a size of at least 4 cm (58 days of culture post-treatment), harvested, and then preserved in formalin. The sex of 100 fish per replicate was determined using the gonadal squash method (see Guerrero and Shelton, 1974). Gonads containing both ovarian and testicular tissue were classified as "intersex". To determine intersex gonads, the percentage of ovarian tissue within the length of the gonad was estimated.

The experimental design was a 2 x 4 factorial with two fish types and four treatments. Treatment means for average weight, length, survival, feed conversion ratio, and percent males were compared by two-way analysis of variance (ANOVA). Feed conversion ratio equals the amount of feed given divided by the weight gain of the fish. Difference

Table 2. Number of Red and Nile tilapia fry female per kg of female and by size class harvested from 4 trials from February to July in Comayagua, Honduras. Data are as means of two replicates per trial.

	No. Fry/kg Female	No. Fry/ Female	No. Fry < 14 mm	No. Fry ≥ 14mm	Mean Water Temperature	Degree-Days to Harvest	Percent Red Fry
TRIAL 1							
Red	1,155	158	32,298	1,250	25	221	74
O. niloticus	1,440	172	36,388	316	25	218	
TRIAL 2							
Red	2,427	297	57,250	750	30	228	77
O. niloticus	2,362	249	54,500	750	30	225	
TRIAL 3							
Red	3,820	506	105,500	2,250	30	232	84
O. niloticus	3,048	336	74,750	1,150	30	231	
TRIAL 4							
Red	3,468	603	134,500	1,500	29	225	73
O. niloticus	3,952	410	93,000	500	31	226	
GRAND MEAN							
Red	2,718	391	82,387 *	1,438 *	29	227	77
O. niloticus	2,701	292	64,659 *	679 *	29	225	

* Values in the same column are significantly different ($P < 0.05$).

among treatments was detected using Fisher PLSD and Sheffe F-test ($p < 0.05$). All statistical analyses were made using a Statview + Graphics program (Feldman et al., 1988).

Results and Disscussion

Phase 1-Reproduction

Minimum and maximum water temperature during the trials ranged from 21.0 to 36.0°C. Cumulative degree-days (Pruess, 1983) per trial ranged from 218 to 232 with a mean of 225.8. Green and Teichert-Coddington (1993) recommend that for maximum production of fry less than 14 mm long, harvest should be between 195 and 220 degree-days. Both RT x RT and Nile tilapia conformed well to these guidelines, producing a total of 329,548 and 258,638 fry less than 14 mm with 0.01% of the total fry production being greater than 14 mm for all four trials (Table 1). Morning dissolved oxygen never fell below 2.5 mg/l. and disease outbreaks did not occur.

The mean survival rate for female RT broodstock was 94.8% and 96.3% for males in all four trials. The mean survival for Nile tilapia was 96.4% for females and 98.3% for males. Teichert-Coddington et al. (1993) suggested that RTxRT brood tilapia may have reduced survival due to their susceptibility to predation and handling; however, in this study red and Nile tilapia broodstock had similar survival (Table 1).

There was a significant difference between mean average weight of females; female average weight also increased with time ($p = 0.01$). Mean average weight for red females was 141.3 g, and 109.9 g for Nile tilapia females. Mean average weight for red males was 276.6 g, and 238.2 g for Nile tilapia males (Table 2). RT x RT and Nile tilapia broodstock had a similar condition factor. A similar number of females was used from trial to trial, but due to weight changes female standing crops differed from trial to trial. The number of fry produced was not correlated to standing crop ($R^2 = 0.18$; $p = 0.12$) over a range of standing crops from 976 kg/ha to 1548 kg/ha (Table 1). Sex ratio did not change from

trial to trial and had no effect on the number of fry produced ($p = 0.37$).

Both brood types had similar fecundities (Table 2), averaging 2,718 fry per kg of female for RT x RT and 2,701 fry per kg of female Nile tilapia. Fecundity was not correlated to female weight in either species when female weights ranged from 122 to 174 g and 104 to 120 g during the four trials for RT x RT and Nile tilapia, respectively ($R^2 = 0.23$; $p = 0.22$). The RT broodfish produced an average of 72.7% red-colored fry, while 27.3% fry had a wild-color pattern. El Gamal (1987) found no difference in number of eggs per kg of female body weight in *O. niloticus* and *O. aureus* over a weight range of 103 to 172 g. No difference in numbers of fry produced per kg of female body weight was also reported by Dazdzie (1970) and Sunusi (1984).

The total number of fry produced differed significantly between trials ($p = 0.002$). Fry number was positively correlated to male:female weight ratio ($R^2 = 0.39$; $p = 0.001$), degree-days ($R^2 = 0.51$; $p = 0.002$), and water temperature ($R^2 = 0.58$; $p = 0.0006$). There was, however, a stronger relationship to fry increase by trial ($R^2 = 0.85$; $p = 0.0001$). Trial 1 had an average water temperature of 25°C, while trials 2, 3 and 4 were conducted at temperatures ranging from approximately 29 to 31°C.

Analysis by trial indicated that fry production was not correlated to male:female weight ratio, female weight, or degree-days, but there was some correlation to male weight ($R^2 = 0.31$; $p = 0.01$). Fry production was not correlated to male or female condition. Guerrero and Guerrero (1985) reported that increase in fry numbers (absolute number) was due to increased size of females, but that was not the case in this study. There was no difference in fecundity by brood type at 30°C.

A possible explanation for the increase in fry production over time may be that territory and social hierarchy of the broodstock were previously established by a group in previous trials. At the start of these trials the brooders had been held in earthen ponds undisturbed for one month. They were then harvested and separated by sex, held in 20 m^3 concrete tanks, and fed a complete ration (30% protein) at 1.5% body weight per day for 10 days. Between trials the broodfish were separated by sex, held for 7 to 10 days, and fed 1.5% body weight per day. During the four trials 95% of the broodfish were restocked. Little (1989) reported improved synchrony of spawning in hapas when spawned females were conditioned in hapas and then again spawned after conditioning.

Phase II: Sex Reversal

The average percentage of males in control groups was 48.3% for *O. niloticus* and 46.0% for red fry. El Gamal (1987) reported that sex ratios of red tilapia were skewed consistently toward males and that sex ratios for normally-pigmented fish were highly variable. Sex ratio for the red strain used in this study was not skewed. The interaction between species and days of feeding did not affect the percentage of males produced. Hormone-treated Nile tilapia and RT x RT fry had similar percentages of males (Table 3). Fry of Nile tilapia were 85, 92, and 82% male after 14, 21, and 28 days of hormone treatment, respectively. RT x RT fry were 85, 83, and 87% male after 14, 21, and 28 days of treatment, respectively. Treatment durations of 14 to 28 days resulted in similar percentage of males for both types of fry. The overall percentage of males produced was lower than expected, particularly for the 21- and 28-day treatments. Berger and Rothbard (1987) produced 97.3 and 99.7% males when fry were fed 17-ethynyltestosterone (17-ET) at a rate of 60 and 120 mg 17-ET/kg for 28 days, respectively. McGeachin et al. (1987) concluded that excessive doses of MT (60-120 mg/kg feed) for 22 days did not produce abnormalities or affect survival or sex reversal in *O. aureus*.

There was no significant difference between the percentage of intersex fish produced in red and *O. niloticus* fry based on the length of treatment period ($p > 0.05$). There were 3, 1 and 0% intersex fish for red fry and 10, 2, and 6% intersex fish for *O. niloticus* fry treated for 14, 21 and 28 days, respectively (Table 3). Watanabe et al. (1991) reported 1.5% intersex fish produced during a hormone treatment of red tilapia which resulted in 94.3 to 98.1% males.

Fry in each hormone treatment received a similar quantity of hormone during the treatment period. Fry received an average of 1.7 µg MT/g fish/d for 28 days. There was a correlation between the percentage of male fry and the quantity of hormone fed fry/d ($R^2 = 0.3$; $p = 0.003$). Pandian and Varadaraj (1988) reported 100% masculinization of *O. mossambicus* fry when fry received 1.5 µg/g fish/d for a duration of 11 days. Varadaraj et al. (1994) found that fry treated with 5 or 10 mg MT/kg diet produced 100% males.

Table 3. Sex composition of red and Nile tilapia fry fed a diet treated with 60 mg 17 α-methyltestosterone/kg diet for 14, 21, and 28 days. Data are based on means of four replicates (100 fish/replicate). No significant differences ($p > 0.05$) were observed among treatments for any parameter.

Species	Duration of Androgen Treatment (d)	% Males	% Females	% Intersex	Total No. of Fish Sexed
Red x Red	0	46	54	0	400
O. niloticus	0	48	52	0	300
Red x Red	14	85	12	3	400
O. niloticus	14	85	5	10	400
Red x Red	21	83	16	1	400
O. niloticus	21	92	6	2	400
Red x Red	28	87	13	0	400
O. niloticus	28	82	10	6	400

Red and Nile tilapia fry fed MT for 14, 21, or 28 days showed no significant difference in mean survival ($p > 0.05$, Table 4). El Gamal (1987) reported a difference in survival between red (*Oreochromis* spp.) and *O. niloticus* and *O. aureus* fry ($p = 0.05$). A comparison of red-colored and wild-colored (black) fry within the red treatments revealed that red fry comprised 73% of the initial population and 69% of the total population at harvest. No significant difference between red and *O. niloticus* fry were found when FCR or growth were compared ($p > 0.05$, Table 4). Wild-colored (black) and red fry within the "red" groups did not differ in length after 28 days of treatment. El Gamal (1987) reported that red fish consistently had a lower average weight gain than their normally pigmented siblings.

Table 4. Average length (mm), mean individual weilght (g), mean net weight per treatment (g), percent survival and food conversion ratio for red tilapia and Nile tilapia fry fed hormone treated feed for 14, 21, and 28 days. Data are means of 4 replicates. No significant differences ($p > 0.05$) were observed among treatments for any parameter.

	Total Length (mm)	Weight Fish (g)	Net Weight (g)	% Survival *	FCR
DAY 14					
Red x Red	20.0	0.2	322	96	0.6
O. niloticus	22.1	0.2	334	84	0.5
DAY 21					
Red x Red	27.0	0.4	524	65	0.8
O. niloticus	27.3	0.4	571	78	0.7
DAY 28					
Red x Red	32.1	0.6	817	69	0.8
O. niloticus	32.7	0.7	979	78	0.8

* Survival to the end of hormone treatment period.

Conclusion

The red color variant of a predominately *O. niloticus* strain had similar fecundity to wild-colored *O. niloticus*. Broodstock survival, fry per kg female, and overall numbers of fry produced were similar. Fry production increased over time but was not correlated to male:female weight ratio, broodstock condition, or female weight. Increase in fry production from trial to trial was possibly related to decreased territorial conflicts due to a social hierarchy established by a group during previous trials.

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