



# AQUACULTURE CRSP 22<sup>ND</sup> ANNUAL TECHNICAL REPORT

## EVALUATION OF GROWTH AND REPRODUCTION CAPACITY OF THREE STRAINS OF NILE TILAPIA, *OREOCHROMIS NILOTICUS*, FOUND LOCALLY IN KENYA FOR USE IN AQUACULTURE

*Tenth Work Plan, New Aquaculture Systems/New Species Research 4B (10NSR4B)  
Final Report*

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### ABSTRACT

Nile tilapia *Oreochromis niloticus* is one of the primary tilapia species being cultured. Much of the stocks being cultured are based on a limited number of collections from the wild, which may differ in their reproductive and growth characteristics. An evaluation of reproductive and growth characteristics of the Egypt, Ivory Coast, Sagana, and Lake Victoria strains of *O. niloticus* was done at Auburn University. Brood fish were stocked into individual 2 m<sup>3</sup> hapas suspended in 20 m<sup>2</sup> concrete tanks. Hapas were checked weekly for successful spawners. Females found holding eggs or sac fry in their mouths were removed and transferred to the hatchery for weighing, counting, and subsequent incubation of eggs and sac fry in 40-L aquaria until hatching. Growth was evaluated at primary and secondary nursery stages. In the secondary nursery stage, growth was studied in outdoor concrete tanks and indoor aquaria on a recirculating system. There were no significant differences ( $P > 0.05$ ) among the four strains in relative fecundity (eggs/g female weight). However, significant differences among strains in spawning and incubation successes were observed. There were no significant differences in growth performance among the strains ( $P > 0.05$ ). However, significant growth differences were observed across production systems at the secondary nursery stage.

The microsatellite variability of the above four strains of *O. niloticus* was described using thirteen primer pairs from *O. niloticus* in amplification reactions. Amplification products were subjected to electrophoresis on 7 % acrylamide gel followed by manual scoring of alleles. Results obtained showed moderate overall strain differentiation with an overall  $F_{ST}$  value of 0.18. All four strains showed some heterozygote deficiency when tested for Hardy-Weinberg equilibrium with observed heterozygosities falling short of expected values. Length of domestication impacted genetic diversity with Ivory Coast strain showing reduced genetic diversity observed in low number of both total and rare alleles.

### INTRODUCTION

Tilapia (family: Cichlidae) are one of the most widely cultured group of fish around the world. They are native to several regions in Africa and the Jordan Valley in the Middle East. They are now widely distributed in the tropical, sub-tropical and some temperate climates around the world through artificial introductions.

Of all the tilapia, Nile tilapia *O. niloticus* has become the main species of culture on account of its fast growth rate, adaptability to a wide range of environmental conditions and high consumer acceptability. As a result of its wide native distribution, many populations exist, offering

potential differences in their reproductive and growth efficiencies, and suitability for aquaculture. The objectives of this study were two phase: 1) to evaluate four strains of Nile tilapia for their suitability for aquaculture relative to each other; and 2) and describe the genetic diversity associated with these strains and how that is related to the domestication history of the strains.

### METHODS AND MATERIALS

In phase 1, reproductive and growth characteristics of four strains of *O. niloticus* were evaluated from May 2002 to July 2003 at the north Auburn Fisheries Research Unit, Alabama Agricultural Experiment Station; Auburn Uni-

versity, Alabama. Facilities included those for breeding, growth comparison and genetic analysis. Breeding units used were 2 x 1 x 1 m hapas suspended in 20 m<sup>2</sup> concrete tanks and 40-L aquaria for egg incubation. The growth comparison units were: (a) circular plastic tanks (1.32 m<sup>3</sup>) for primary nursery growth phase, (b) rectangular concrete tanks (area 20 m<sup>2</sup>, depth 0.75 m) for secondary nursery growth phase and (c) 40-L aquaria for indoor secondary nursery growth comparison.

### Fish Used In the Study

Four populations (strains) of *O. niloticus* of varying geographical origins and domestication histories were used. Ivory Coast strain was introduced to Auburn University from Fortaleza, Brazil in 1974 in a batch of 100 fish. Ancestors of the strain (100-200 fish) were introduced to Fortaleza from Bouake, Ivory Coast in 1971 and reproduced in Brazil (Lovshin and De Silva, 1975). Trewavas (1983) stated that the stock used in pond culture in Bouake, Ivory Coast, was from the tributaries of the Niger and Lake Volta in the northern part of that territory. Egypt strain of *O. niloticus* used at Auburn University was initially collected from the Ismailia canal of the Nile River, about 75 km northeast of Cairo and introduced to Auburn University in May 1982. Individuals of the founder stock averaged 45g and consisted of 20 males and 66 females. Sagana and Lake Victoria strains were introduced to Auburn University in March 2002 from Kenya. The Sagana strain originated from Lake Turkana and was introduced to Baobab farm, Kenya in the early 1980s. It was then introduced to Sagana Fisheries Research Station from the Baobab farm in 1994. It is a subspecies of Nile tilapia classified by Trewavas (1983) as *O. n. vulcani*. The stock that was introduced to Auburn University comprised of only 35 fingerlings weighing about 10 g each. Fish of the Lake Victoria strain were the first generation offspring of brood stock that had been obtained from Lake Victoria four months prior to their introduction to Auburn University, and thus had the shortest domestication history. The introduction was comprised of 240 three weeks old fry. Brooders from all strains were held in a common recirculating system and managed similarly until their use in the spawning experiments.

### Reproduction Phase

First Trial: A total of 130 Ivory Coast and 108 Egypt brood stocks with average weights 414.7 g and 519.1 g, respectively, were available for use. The fish were stocked into 2 m<sup>3</sup> hapas suspended in 20 m<sup>2</sup> outdoor concrete tanks for breeding on 30 May 2002 using 1 tank per strain and four replicate hapas per tank. The stocking density was 3 males to 7 females per hapa. The breeding period lasted 4 weeks. The hapas were checked weekly for females with eggs or fry in their mouths. Females with eggs or fry were transferred to the hatchery

where eggs or fry were gently removed from the mouths of females for counting and weighing. Brood females were weighed and lengths measured. Each female was stocked into separate 40 L aquaria along with her eggs or fry. Females were left to pick up their brood without any further intervention. The number of females that picked up their brood and continued with egg incubation and fry development was recorded after 24 hours. Incubation period lasted for 5-7 days. The fry were manually counted at the swim-up stage and the spawners taken back to the hapas. Percent spawning success (number of females that spawned out of the total that were given the chance to spawn), percent females that picked up brood after transport to the hatchery, and percent survival of fry to swim-up stage were computed and recorded.

Second Trial: Ivory Coast, Sagana and Lake Victoria strains were evaluated and female fish averaged 63.5g, 137.2 g, and 98.4 g, respectively. Males of comparable weights were used. Fish were stocked into hapas at a density of 3 males to 7 females on 18 July 2002. The breeding period lasted 8 weeks. The aforementioned procedures for trial 1 were used in this trial as well.

Third Trial: Egypt, Sagana and Lake Victoria strains were evaluated in this trial. Females with average weights of 110.3, 128.5 and 148.9 g, respectively, were stocked into hapas with males of comparable sizes at the ratio of 3 males to 7 females. Hapas were checked for successful spawners at weekly intervals. The breeding period started on 29 May 2003 and lasted 6 weeks.

### Growth Phase

#### Primary Nursery

Growth was evaluated in two stages: (1) primary nursery phase and (2) secondary nursery phase. In the primary nursery phase, sets of 300 fry from individual spawns that hatched almost at the same time ( $1 \pm 0.5$  d) were stocked into 1.32 m<sup>3</sup> static water circular fiberglass tanks at 0.23 fry/L. Each strain was represented by four sets of fry from four individual females stocked into separate tanks. Three primary nursery studies were conducted. In the first, Egypt and Ivory Coast strains were compared while in the second and third studies, Ivory Coast and Sagana strains, and Egypt and Victoria strains were compared, respectively.

The fry were fed an Aquamax starter diet-5D01 containing 50% crude protein for 30 days. Feeding rates were adjusted daily based on the assumed total number of fry in the tank and the average weight per fish as calculated from the known or estimated length (Popma and Green, 1990). The daily ration was divided into 4 portions and delivered four times a day using belt feeders. At the end of 30 days, the fish were harvested and total number, total weight and total fish length in each fiberglass tank

were recorded.

### Secondary Nursery

The fish (Egypt and Ivory Coast) harvested in the primary nursery phase were used. The first trial of this phase was conducted in (1) indoor recirculating system stocking 50 fish/ 40L aquarium using 4 replicates per strain and, (2) an outdoor setting where fish were stocked into 20 m<sup>2</sup> concrete tanks at 100 fish/tank (5 fish/m<sup>2</sup>) with 6-8 replicates per strain. The latter group was further sub-divided into two groups; fertilized and fed regimes.

Fish in the recirculating system and outdoor tanks (fed treatment), were fed an Aquamax fingerling starter diet-5D03. The daily ration of 5 % body weight was divided into 2 feedings and delivered at 0900 h in the morning and 1500 h in the afternoon. Fingerlings were sampled every two weeks and feeding rates adjusted accordingly. The tanks in the fertilized treatment received weekly applications of moist cow manure, added at the equivalent of 500 kg (dry wt.)/ha/wk.

The second trial was done only in the outdoor tanks and only the fertilization treatment was applied. The Egypt and Ivory Coast fish harvested in the second trial of primary nursery phase were stocked in 20 m<sup>2</sup> (15 m<sup>3</sup>) concrete tanks at a density of 100 fish/tank (5 fish/m<sup>2</sup>, 0.01 fish/L) and raised for 60 days. At the end of the nursery period, percent survival, yield, average fish weight and length were determined for each tank.

### **Data Analysis -- Aquaculture Phase**

Data analysis was done using SAS v 8 (SAS Institute, 2001) statistical package. A two-sample t-test was used to test the difference of the means between two strains while one-way analysis of variance was used to test the difference of the means of more than two samples. Simple linear regression analysis was used to assess the relationship between several variables.

### **Genetic Evaluation**

Blood samples were collected from individual fish and placed into 15 ml tubes containing DNA extraction buffer and freshly added proteinase K (0.1 mg/ml). Thirty samples were taken from each of the four strains and transferred to the molecular genetics lab where they were stored at room temperature until DNA isolation. DNA was isolated using the Puregene DNA Isolation Kit (Gentra Systems, Minneapolis, MN). Thirteen microsatellite DNA primer sequence pairs, selected from earlier work done on Nile tilapia microsatellites by Lee and Kocher (1996), were obtained from the National Center for Biotechnology Information (NCBI) gene bank database under the following names:(UNH-004, UNH-005, UNH-006, UNH-007, UNH-008, UNH-009, UNH-159,

UNH-216, UNH-231, UNH-144, UNH-156, UNH-188 & UNH-132), and used in this experiment. Polymerase chain reactions (PCR) were carried out in 10  $\mu$ L reaction volume containing 100 ng/ $\mu$ L of each primer, and 50 ng/ $\mu$ L template genomic DNA. After amplification reactions were complete, the samples were denatured for 4 minutes prior to loading into a LI-COR DNA Automatic Sequencer (LI-COR Inc. Lincoln, Nebraska) for electrophoretic analysis.

Genotypes were obtained by manually scoring the bands depending on distance traveled in the gel using 50-700 base pair marker as a guide. The scored microsatellite data were fed into GENEPOP Version 3.1d, March 1999 and POPGENE version 1.32, December 2000 software packages for analysis of population characteristics. The tests performed included; Hardy-Weinberg Exact tests (heterozygote excess or deficiency), and population differentiation (genotypic and allelic distributions across populations).

Genetic diversity was characterized by observed heterozygosity  $H_{obs}$ , expected heterozygosity  $H_{exp}$ , the number of alleles and unique alleles per locus per population, and the mean number of alleles per locus. Deviations from Hardy-Weinberg Equilibrium were examined for each population at each locus by calculating Wright's inbreeding coefficient  $F_{IS}$  according to Weir and Cockerham (1984) and using Fisher's exact test with GENEPOP. Pairwise and overall strain differentiation were examined by calculating the  $F_{ST}$  values according to Weir and Cockerham (1984) using GENEPOP. A dendrogram based on Nei's (1978) genetic distance using UPGMA method modified from NEIGHBOR procedure of PHYLIP Version 3.5 was obtained using POPGENE software.

## **RESULTS**

### **Reproductive Characteristics**

In the first trial, Egypt and Ivory Coast strains were not significantly different in the production of eggs or sac fry/g female body weight, total fry/kg female stocked and percent survival of fry to swim-up stage (Table 1,  $P > 0.05$ ). Notable differences in percent spawning success and percent number of females that picked up their brood after transport to the hatchery were observed. Ivory Coast strain had 45.5 % and 91.3 % success in spawning, and number of females that picked up brood in the hatchery, respectively, while Egypt strain only had 36.9 % and 55.0 % success for the same characteristics (Table 1). In the second trial using Ivory Coast, Sagana and Lake Victoria strains, there were significant differences ( $P < 0.05$ ) in all the traits considered: percent spawning success, number of eggs or fry/g female body weight, percent number of females that picked up brood in the hatchery, percent survival to swim-up stage, and total fry/kg female stocked (Table 1). In general the

Lake Victoria strain reproduced less successfully. In the third trial using Egypt, Sagana and Lake Victoria strains, there were differences in mean egg or sac fry production/g body weight of female and total fry/kg female stocked ( $P < 0.05$ ). Egypt strain had significantly higher fecundity and total fry/kg female stocked than Sagana and Victoria strains (Table 1). The three strains showed little differences in percent number of females that picked up brood in the hatchery (Table 1).

### Primary Nursery Characteristics

There was little variability in fry growth characteristics (average lengths, average weights and survival,) among three strains of *O. niloticus* after a 30-day rearing period (Table 2). However, the Ivory Coast strain exhibited more variability in fry survival compared to the Egypt strain (Table 2). In general, the growth rates were quite similar between the strains evaluated in each of the three trials. Average water temperature and dissolved oxygen were  $28.8 \pm 1.1^{\circ}\text{C}$  and  $5.14 \pm 3.51\text{ mg/L}$  respectively.

### Secondary Nursery Characteristics

There were no significant differences observed in growth of fingerlings among strains of *O. niloticus* in either of the two trials when cultured under similar conditions. However, in Trial 1, fish in fed regimes grew better and produced higher yields than the ones in the fertilized regime (Table 3). In the fed regimes, fish in the indoor recirculating system produced higher yields/ $\text{m}^3$  than those in outdoor concrete tanks, even though the reverse was true for average weights at harvest. Mean percent survival was similar for the two strains in all production systems.

### Genetic Characteristics

#### Microsatellite Locus Variability

Nine loci out of the thirteen used produced amplification products. All nine loci were polymorphic with the total number of alleles per locus ranging from 4 to 15 with an average of 8.3 alleles per locus (Table 4). There was on average, a total of 43 alleles per strain for the samples considered in this study (Table 5). Out of thirty-six tests (9 loci, four strains) for Hardy-Weinberg equilibrium, 10 significant deviations were observed (Tables 6 and 7). Four of them were observed in the Egypt strain while the Ivory Coast, Sagana, and Victoria strain had two each (Table 6). Six of the ten deviations had positive  $F_{IS}$  values (heterozygote deficiencies) while four had negative  $F_{IS}$  values (heterozygote excess). The average heterozygosity for all the nine loci across the four strains was estimated to be  $0.592 \pm 0.1$

#### Microsatellite Variability Among Strains

All the four strains of *O. niloticus* showed some deviation from Hardy-Weinberg expectations with observed heterozygosities falling short of expected values (Table 4). Strains ranged in observed heterozygosity from lowest in Ivory Coast strain ( $H = 0.575$ ) to highest in the Victoria strain ( $H = 0.667$ ). Overall mean observed heterozygosity for the four strains was 0.622 while mean expected heterozygosity was 0.643 (Table 5).

#### Population Differentiation

The pair wise  $F_{ST}$  values between strains showed varying levels of population differentiations. In general, some strains showed more differentiation between them than others. Differentiation between Ivory Coast and Sagana strains was the greatest (0.2372) while the least differentiation level was between Sagana and Victoria strains (0.0972). Differentiation level between Ivory Coast and Victoria strains of 0.2127 was close to that between Ivory Coast and Sagana strains. Egypt strain was closest to Ivory Coast (0.119) strain followed by Victoria (0.1683). The overall  $F_{ST}$  value for all the nine loci was 0.18 indicating a moderate level of overall strain differentiation.

#### Phyletic Relationships Among Strains

Phyletic relationships between Egypt, Ivory Coast, Sagana, and Victoria strains of *O. niloticus* are shown in Figure 1. The dendrogram groups the four into two clusters: Egypt and Ivory Coast in one cluster and Victoria and Sagana in the other.

## DISCUSSION

### Reproductive Characteristics

Egypt and Ivory Coast strains were similar the first trial of this study as to relative fecundity and percent survival of fry to swim-up stage. The mean fecundities of 4.07 and 3.07 eggs/g female body weight obtained in the first trial of this study for Egypt and Ivory Coast strains respectively, are smaller in magnitude compared to 11.13, 10.56 and 11.96 eggs/g female body weight for Egypt, Ghana and Ivory Coast strains, respectively, reported by Smitherman et al. (1988). Inbreeding could be responsible for the observed reduced relative fecundity in this study in comparison to that of Smitherman et al. (1988) for Ivory Coast and Egypt strains. The present study was done 7-8 generations later than that of Smitherman et al. (1988) using the same Egypt and Ivory Coast strains. However, the average weight of females used also differed and may have been a factor as well.

In the second trial, Ivory Coast, Sagana and Victoria strains were different ( $P < 0.05$ ) in % spawning success, number of eggs/g female body weight, % incubation success, and % survival of fry to swim-up stage. These

differences may not be real given the variability of brood fish used with regard to readiness to spawn, age, and size at the beginning of the experiment. Ivory Coast strain fish, though smallest in size, were older than the other two and more mature (11 months old). The Sagana and Victoria strains were 8 and 5 months old respectively. It is however, worth noting that under good growth conditions *O. niloticus* will reach sexual maturity in farm ponds at an age of 5 to 6 months or even less (Fitzsimmons, 1997; Popma and Masser, 1999).

Spawning success percentages achieved in the first trial of reproduction in this study ranging between 36.9 – 45.5 % are lower than those reported by Singh (1988) of between 47% and 63% for Egypt and Ghanaian strains of *O. niloticus*. The Egypt and Ivory Coast strains evaluated in first reproductive trial showed no difference in total fry/kg female stocked. However, significant differences in total fry/kg female stocked were observed within the Egypt and Ivory Coast strains across trials. This is attributable to the differences in females sizes used in those trials. Females used in Trial 1 weighed over 300 g, while those used in trials 2 and 3 weighed 100 g and below. Percent female success in picking up brood after transport to the hatchery impacted total fry/kg female stocked in trials 2 and 3 where the Ivory Coast and Egypt strains, respectively, produced more fry than the Sagana and Victoria strains. The influence was, however, not as clear in Trial 1, which exhibited high variability in total fry produced/kg female stocked per hapa.

### Growth Characteristics

Egypt and Ivory Coast strains of *O. niloticus* evaluated in the first trial of this study were, in general, similar in their growth performances during both primary and secondary nursery. This was true for all the production systems that were used. Bolivar et al. (1993) obtained similar growth rates among seven strains of the eight Nile strains tested. Khater (1985) and Jayaprakas et al. (1988) found significant differences in growth performance of Egypt, Ghana and Ivory Coast strains.

There was no genotype-environment interaction evident, as the two strains (Egypt and Ivory Coast) demonstrated no growth advantage in any of the three (indoor aquaria with feed, outdoor tanks with feed or fertilizer) production systems used. Production systems influenced yields and production, but strains did not. In a growth study using ponds, cages and tanks, and high and low selected lines of *O. niloticus*, Abucay and Mair (2000) also observed no genotype-environment interaction.

### Genetic Characteristics

The four populations evaluated did differ genetically. Ten out of 36 tests for Hardy-Weinberg equilibrium showed significant deviations (Table 6). The cause(s)

for these deviations are unknown. Six of these deviations were heterozygote deficiencies (positive  $F_{IS}$  values) while four were heterozygote excesses (negative  $F_{IS}$  values). Out of the ten deviations, the Egypt strain had four while the Ivory Coast, Sagana and Victoria strains had two each. It should be noted that the Egypt strain had heterozygote excesses at two loci and heterozygote deficiencies at two loci. The Ivory Coast strain had only heterozygote deficiencies at two loci while the Sagana and Victoria strains had heterozygote excesses at one locus and heterozygote deficiencies at two loci, respectively. Deviations from Hardy-Weinberg equilibrium, both positive and negative, can arise from a small number of broodstock (Ward et al. 2003). Heterozygote deficiencies can be attributable to other phenomena including inbreeding, population admixture (the Wahlund effect) or the presence of nonexpressed (null) alleles (Ward et al. 2003). In this study the Ivory Coast strain showed the highest overall value of heterozygote deficiency ( $F_{IS}$  0.427), the Egypt and Sagana strains showed intermediate values ( $F_{IS}$  0.177 and 0.161, respectively) while the Victoria strain had the lowest value ( $F_{IS}$  0.062). This observation is consistent with strain history and potential inbreeding. The Ivory Coast strain at Auburn University is the most inbred while the Lake Victoria strain with its short domestication history is the least inbred.

The number of alleles found in each strain (population) shows its genetic variability. Examining private alleles (alleles observed in only a single strain) reveals their uniqueness. The number of private alleles in the four strains varied with the Victoria strain having 9 while the Sagana, Egypt and Ivory Coast strains had 5, 4, and 2, respectively (Tables 6 and 7). This observation probably reflects loss of genetic diversity in Ivory Coast strain through inbreeding due to founder effect and genetic drift. The Lake Victoria strain, which was recently taken from the wild, had the highest number of private alleles and heterozygosity level, hence the greatest level of genetic diversity.

The highest levels of pair wise strain differentiation was seen between Ivory Coast and Sagana and Ivory Coast and Victoria strains ( $F_{ST}$  of 0.2372, 0.2127), respectively. The Ivory Coast being from a different drainage than the Sagana and Victoria strains as well as having a longer domestication history contributes to the observed population differentiation. Lowest strain differentiation was observed between Victoria and Sagana ( $F_{ST}$  = 0.0972). This is less surprising given their more similar origins. The dendrogram based on Nei's (1978) genetic distance grouped Egypt and Ivory Coast strains in one cluster and Sagana and Victoria strains in another cluster (Figure 1). The genetic closeness of the Sagana and Victoria strains is understandable given their similar origin as mentioned earlier. The observation that the Egypt and Ivory Coast strains are also genetically close is somehow unexpected given their different geographical origins.

There are two possible reasons for this observation. First, domestication in the same farm might have led to an accidental mixing of the two strains along the way, but this possibility is ruled out by the presence of private alleles in both strains. The presence of private alleles unique to each strain suggests that two distinct populations have been maintained. A second reason may be inbreeding and selective pressures associated with domestication (Dunham et al., 2001) may have considerably changed gene frequencies of the two strains when in held in similar environments.

### CONCLUSIONS

Although the four populations of tilapia were genetically distinct, they were similar in their reproduction and growth through secondary nursery characteristics. Based on the traits considered, no one strain could be considered superior to the other. No detrimental effects from inbreeding or domestication history were apparent. However, domestication does result in a loss of gene frequency, which might make a given strain less adaptable to new production techniques. Microsatellite analysis proved to be an effective method for genetically characterizing strains of fish both in terms of their uniqueness as well as their domestication history.

### ANTICIPATED BENEFITS

The above study supports the concept that domestication and inbreeding of established lines of Nile tilapia does not necessarily result in inferior lines of fish (at least through secondary nursery). Fish stocks currently being cultured can be produced effectively without having to introduce new strains to a given site. In the case of Nile tilapia and its culture within its native range, it may be possible to produce fish using strains that are native to the area, versus introducing a more established and more accessible strain available from elsewhere. Biodiversity can be maintained without limiting aquaculture development. Protocols that are practical to implement in the field can be used to evaluate similar strains of fish before widespread distribution plans are implemented.

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Table 1. Reproductive characteristics of Egypt, Ivory Coast, Sagana and Victoria strains of Nile tilapia *Oreochromis niloticus*.

Parameters	Egypt	Ivory Coast	Sagana	Victoria
<b>First Trial</b>				
Female average weight (g)	380.1 ± 79.5 <sup>a</sup>	345.2 ± 55.1 <sup>a</sup>		
Percent Spawning success	36.9	45.5		
No. of eggs/g female that spawned	4.1 ± 1.3	3.1 ± 1.2		
Percent females incubating eggs	55.0 <sup>a</sup>	91.3 <sup>b</sup>		
Percent Survival of fry to swim-up	63.5 ± 38.9 <sup>a</sup>	81.3 ± 22.2 <sup>a</sup>		
Total fry/kg female stocked	702 ± 225 <sup>a</sup>	999 ± 274 <sup>a</sup>		
<b>Second Trial</b>				
Female average weight (g)		63.5 ± 22.6 <sup>a</sup>	137.2 ± 24.3 <sup>b</sup>	98.4 ± 11.2 <sup>c</sup>
Percent Spawning success		48.2 <sup>a</sup>	28.7 <sup>b</sup>	11.6 <sup>c</sup>
No. of eggs/g female		11.4 ± 5.7 <sup>a</sup>	6.2 ± 3.9 <sup>b</sup>	4.3 ± 3.2 <sup>b</sup>
Percent females incubating eggs		59.1 <sup>a</sup>	36.8 <sup>b</sup>	5.3 <sup>c</sup>
Percent Survival to swim-up		41.4 ± 37.9 <sup>a</sup>	21.5 ± 32.7 <sup>b</sup>	3.00 ± 13.1 <sup>c</sup>
Total fry/kg female stocked		2346 ± 494 <sup>a</sup>	413 ± 184 <sup>b</sup>	106 ± 93 <sup>b</sup>
<b>Third Trial</b>				
Female average weight (g)	110.3 ± 22.08 <sup>a</sup>		128.5 ± 52.37 <sup>a</sup>	148.9 ± 39.61 <sup>b</sup>
Percent Spawning success	57.5 <sup>a</sup>		44.5 <sup>b</sup>	31.8 <sup>c</sup>
No. of eggs/g female	4.2 ± 2.2 <sup>a</sup>		2.1 ± 0.88 <sup>b</sup>	2.5 ± 0.89 <sup>b</sup>
Percent females incubating eggs	71.8		66.7	64.7
Percent Survival to swim-up	78.66 ± 12.64		84.14 ± 11.35	68.9 ± 19.62
Total fry/kg female stocked	1501 ± 257 <sup>a</sup>		653 ± 387 <sup>b</sup>	312 ± 53 <sup>b</sup>

Values with different superscript letters a, b & c within rows are significantly different at P < 0.05.

Table 2. Comparative growth characteristics of three strains of Nile tilapia *Oreochromis niloticus* during a primary nursery stage of 30 days when stocked at 170 fish/m<sup>2</sup> and fed a commercial feed at 10 % body weight/day.

Parameters	Egypt	Ivory Coast	Sagana	Victoria
<b>First Trial</b>				
Initial length (mm)	8.75 ± 0.37 <sup>a</sup>	8.28 ± 0.10 <sup>a</sup>		
Final average length (mm)	51.0 ± 1.67 <sup>a</sup>	51.8 ± 1.74 <sup>a</sup>		
Final average weight (g)	2.55 ± 0.21 <sup>a</sup>	2.83 ± 0.30 <sup>a</sup>		
Yield (kg/m <sup>3</sup> )	0.81 ± 0.10 <sup>a</sup>	0.88 ± 0.09 <sup>a</sup>		
Percent Survival	93.8 ± 5.00 <sup>a</sup>	76.2 ± 39.4 <sup>a</sup>		
<b>Second Trial</b>				
Initial length (mm)		8.16 ± 0.13 <sup>b</sup>	8.23 ± 0.11 <sup>b</sup>	
Final average length (mm)		47.8 ± 1.81 <sup>b</sup>	50.9 ± 2.5 <sup>b</sup>	
Final average weight (g)		2.13 ± 0.22 <sup>b</sup>	2.46 ± 0.43 <sup>b</sup>	
Yield (kg/m <sup>3</sup> )		0.69 ± 0.10 <sup>bx</sup>	0.81 ± 0.11 <sup>by</sup>	
Percent Survival		95.3 ± 5.30 <sup>b</sup>	95.4 ± 1.00 <sup>b</sup>	
<b>Third Trial</b>				
Initial length (mm)	8.81 ± 0.14 <sup>c</sup>			8.29 ± 0.26 <sup>c</sup>
Final average length (mm)	54.6 ± 3.31 <sup>c</sup>			51.6 ± 1.04 <sup>c</sup>
Final average weight (g)	2.55 ± 0.32 <sup>c</sup>			2.21 ± 0.29 <sup>c</sup>
Yield (kg/m <sup>3</sup> )	0.54 ± 0.05 <sup>c</sup>			0.49 ± 0.06 <sup>c</sup>
Percent Survival	93.5 ± 4.52 <sup>c</sup>			98.1 ± 0.51 <sup>c</sup>

Rows with different superscript letters a, b, c show significant difference at 0.05 level while those with superscript letters x, y, z are significant at 0.1 level.

Table 3. Secondary nursery growth values for two strains of *Oreochromis niloticus* reared in various production systems and food regimes for 60 days where fertilized tanks received cow manure at 500 kg/ha/wk, outdoor fed tanks received 5 % body weight commercial feed/day and indoor recirculating system received 3-5 % body weight commercial feed/day.

Parameters	Trial 1		Trial 2	
	Egypt	Ivory Coast	Egypt	Ivory Coast
<u>Yield at Harvest</u>				
Fertilized, outdoor tanks (kg/ha)	1067 ± 356 <sup>a</sup>	982 ± 273 <sup>a</sup>	869 ± 83 <sup>a</sup>	974 ± 281 <sup>a</sup>
Fed, outdoor tanks (kg/ha)	4095 ± 1494 <sup>b</sup>	5025 ± 242 <sup>b</sup>		
Fed, indoor recirculating system (kg/m <sup>3</sup> )	41.0 ± 2.3 <sup>c</sup>	43.6 ± 3.8 <sup>c</sup>		
<u>Average weight at harvest (g)</u>				
Fertilized, outdoor tanks	21.8 ± 7.3 <sup>a</sup>	21.0 ± 6.5 <sup>a</sup>	18.7 ± 2.2 <sup>b</sup>	19.8 ± 5.8 <sup>b</sup>
Fed, outdoor tanks	87.9 ± 23.1 <sup>b</sup>	103.2 ± 3.9 <sup>b</sup>		
Fed, indoor recirculating system	36.1 ± 2.6 <sup>c</sup>	36.5 ± 2.4 <sup>c</sup>		
<u>Percent Survival</u>				
Fertilized, outdoor tanks	98.0 ± 2.0 <sup>a</sup>	94.0 ± 2.8 <sup>a</sup>	93.5 ± 6.56 <sup>c</sup>	98.5 ± 2.38 <sup>c</sup>
Fed, outdoor tanks	91.3 ± 13.3 <sup>b</sup>	97.3 ± 1.53 <sup>b</sup>		
Fed, indoor recirculating system	91.0 ± 3.8 <sup>c</sup>	95.5 ± 5.3 <sup>c</sup>		
<u>Food conversion ratio (FCR)</u>				
Fed, outdoor tanks	1.26 ± 0.15 <sup>a</sup>	1.2 ± 0.03 <sup>a</sup>		
Fed, indoor recirculating system	1.49 ± 0.07 <sup>b</sup>	1.46 ± 0.08 <sup>b</sup>		
<u>Production (kg/ha/day)</u>				
Fertilized, outdoor tanks	15.68 ± 5.98 <sup>a</sup>	14.09 ± 4.68 <sup>a</sup>	12.89 ± 1.21 <sup>d</sup>	14.27 ± 4.34 <sup>d</sup>
Fed, outdoor tanks	66.14 ± 24.97 <sup>b</sup>	81.48 ± 3.98 <sup>b</sup>		
Fed, indoor recirculating system (kg/m <sup>3</sup> /day)	0.64 ± 0.03 <sup>c</sup>	0.67 ± 0.06 <sup>c</sup>		
<u>Growth (g/fish/day)</u>				
Fertilized, outdoor tanks	0.32 ± 0.12 <sup>a</sup>	0.30 ± 0.11 <sup>a</sup>	0.28 ± 0.03 <sup>a</sup>	0.29 ± 0.09 <sup>a</sup>
Fed, outdoor tanks	1.60 ± 0.33 <sup>b</sup>	1.68 ± 0.06 <sup>b</sup>		
Fed, indoor recirculating system	0.56 ± 0.04 <sup>c</sup>	0.57 ± 0.04 <sup>c</sup>		

Rows with different superscript letters a,b,c,d for the same trial are significantly different at 0.05 level.

Table 4. Measures of Genetic Variability for the Nine Microsatellite Loci of *Oreochromis niloticus*.

Locus	Average Heterozygosity	Total Number Of Alleles	Average Number of Alleles per strain
UNH 005	0.5418	5	3.00
UNH 006	0.6366	7	5.00
UNH 008	0.5177	9	4.25
UNH 009	0.6786	12	6.50
UNH 159	0.7073	15	5.75
UNH 156	0.4485	10	7.00
UNH 144	0.4651	4	3.25
UNH 132	0.6489	5	4.25
UNH 188	0.686	8	4.50
Average	0.5923	8.3	4.83

Table 5. Number of Loci, Allele Number, Private Alleles, and Genetic Heterozygosity for four strains of *Oreochromis niloticus*.

Strain	Loci	Allele Number	Alleles per locus	Private Alleles	Observed Heterozygosity	Expected Heterozygosity
Egypt	9	42	4.7	4	0.621	0.64
Ivory Coast	9	37	4.1	2	0.575	0.607
Sagana	9	46	5.1	5	0.623	0.642
Lake Victoria	9	47	5.2	9	0.667	0.682
Average	9	43	4.8	5	0.622	0.643

Table 6. Wright's Inbreeding Coefficient  $F_{IS}$  for 9 microsatellite loci for four strains of *O. niloticus* computed according to Weir and Cockerham (1984).

Strain Locus	Egypt's Fwc(is)	Ivorycoast's Fwc(is)	Sagana's Fwc(is)	Victoria's Fwc(is)
unh005	-0.606*	-0.217	-0.099	0.065
unh006	0.127	0.289*	-0.011	0.065
unh008	0.318*	-0.111	0.492*	0.209
unh009	-0.123	0.201	0.153	-0.043
unh159	0.720*	0.431*	0.019	0.313*
unh156	-0.077	-0.168	-0.035	-0.189
unh144	-0.430*	-0.108	-0.174	0.1
unh132	0.05	0.105	-0.263*	-0.471*
unh188	0.198	0.005	0.079	0.013
Overall	0.177	0.427	0.161	0.062

\* Shows significant deviation from Hardy-Weinberg equilibrium.

Table 7. Nile tilapia allele frequencies,  $F_{IS}$  values and sample sizes (n = number of fish) in four strains (Egypt, Ivory Coast, Sagana, and Victoria).

Locus	Allele	Egypt	Ivory Coast	Sagana	Victoria
UNH005	142	0.431	0.267	0.7	0.196
	145	0.569	0.667	0.14	0.326
	148	-	-	0.16	0.478
	151	-	0.067	-	-
	Fis	-0.606*	-0.217	-0.099	-0.065
	n	29	15	25	23
UNH006	245	-	-	0.056	0.088
	248	0.026	-	0.222	0.294
	251	0.316	-	0.556	0.206
	254	0.263	0.2	0.056	0.235
	260	0.211	0.75	-	0.088
	263	0.158	0.05	0.111	-
	264	0.026	-	-	-
	Fis	0.127	0.29*	-0.011	0.065
	n	19	10	9	17
UNH008	200	-	-	-	0.227
	206	0.667	-	-	0.182
	209	-	-	-	0.227
	212	-	0.125	0.429	0.318
	215	0.017	-	-	-
	218	0.1	0.875	0.107	0.045
	222	0.117	-	0.357	-
	225	0.067	-	0.107	-
	228	0.033	-	-	-
	Fis	0.318*	-0.111	0.492*	0.209
	n	30	12	14	11
UNH009	225	0.391	0.357	-	0.024
	231	0.413	0.25	0.077	0.071
	237	0.022	0.143	-	0.19
	240	-	-	0.269	-
	243	-	0.36	-	-
	246	-	-	0.192	0.405
	249	-	-	-	0.143
	252	-	0.071	0.038	0.119
	255	-	-	308	-
	258	-	-	0.077	0.024
	264	0.174	0.107	-	0.024
	270	-	0.036	0.038	-
Fis	-0.123	0.201	0.015	-0.043	
	n	23	14	13	21
UNH159	210	0.038	0.067	-	-
	214	-	-	0.105	-
	216	-	-	-	0.028
	219	-	-	-	0.167
	234	0.192	-	-	-
	236	-	-	0.211	-
	238	0.404	0.5	-	-
	244	-	-	-	0.139
	246	-	-	0.105	0.028
	248	-	-	0.211	0.083
	250	-	-	0.184	0.139
	252	-	-	0.132	0.111
	258	-	-	-	0.222
	260	-	-	0.053	0.083

Table 7. Continued.

Locus	Allele	Egypt	Ivory Coast	Sagana	Victoria
	262	0.365	0.433	-	-
	Fis	0.72*	0.431*	0.019	0.313*
	n	26	15	20	18
UNH156	130	0.188	0.25	0.06	0.109
	136	0.042	0.042	-	-
	141	0.104	0.083	0.02	-
	145	0.125	0.125	0.02	-
	180	-	-	0.22	0.283
	190	-	-	0.58	0.5
	198	0.271	0.25	0.06	0.109
	200	0.042	0.042	-	-
	206	0.104	0.083	0.02	-
	210	0.125	0.125	0.02	-
	Fis	-0.077	-0.168	-0.035	-0.189
	n	24	12	25	23
UNH144	140	0.05	0.206	0.053	-
	142	0.65	0.735	0.763	0.559
	144	0.3	0.059	0.132	0.324
	148	-	-	0.053	0.118
	Fis	-0.43*	-0.108	-0.174	0.1
	n	30	17	19	17
UNH132	112	0.133	0.033	-	-
	114	0.55	0.4	0.25	0.583
	115	0.233	0.2	0.25	0.333
	116	0.067	0.267	0.375	0.083
	118	0.17	0.1	0.125	-
	Fis	0.05	0.105	-0.263*	-0.471*
	n	30	15	4	6
UNH188	176	-	-	0.022	-
	178	0.241	0.423	0.065	0.304
	180	0.31	0.115	0.152	0.022
	182	0.345	0.462	0.217	0.13
	182	0.103	-	0.13	0.435
	190	-	-	0.413	0.109
	Fis	0.198	0.005	0.079	0.013
	n	29	13	23	23

$F_{IS}$  values with \* show significant deviation from Hardy-Weinberg Equilibrium.

Figure 1. A dendrogram based on Nei's (1978) genetic distance showing phyletic relationships among four strains (Egypt, Ivory Coast, Sagana and LakeVictoria) of *O. niloticus*.

