Nile Tilapia Broodstock Selection, Seed Quality and Density-Dependent Growth in the Philippines

Quality Seedstock Development/Experiment/09QSD01NC

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ABSTRACT

To support the growing tilapia industry, there is a need to provide year-round, high-quality seed that can be widely distributed at reasonable costs to tilapia farmers. This can be achieved through better broodstock quality, increased hatchery development and enhanced technologies for consistently high-quality seed and fingerling production. Through a series of four studies we assessed if physiological and/or behavioral responses to stress can be used in the selection of broodstock with reproductive advantage in Nile tilapia, examined the effect of broodstock social condition on seed production and fingerling growout performance of tilapia, and evaluated the effects of stocking density on fingerling growth, gene expression of insulin-like growth factor-1 (IGF-1), and stress responsiveness in nursery hapas. In the first study, we investigated whether the outcomes of competition for social dominance among Nile tilapia individuals can be predicted by evaluating the duration of appetite inhibition (DAI) or the feeding response score (FRS) after transfer to isolation. Seventeen fish (70.93%) of the 24 that became dominant have shorter DAI compared to that of their conspecifics (Binomial test, $P = 0.03$). This indicated that social dominance can be predicted using the DAI of the fish during isolation. Reduced growth rate of both dominant and subordinate fish, a well-described physiological end result of social stress, was observed one day after the social interaction. The significantly greater weight loss ($P < 0.01$) in subordinate fish (2.88 ± 0.21 g) compared to dominant fish (2.11 ± 0.19 g) a day after the establishment of social hierarchy was mainly attributed to behavioral differences such as appetite rather than to differences in physical activities. The second phase of this study was similar to the first except we evaluated FRS over a 10-day isolation period as a predictor of social status. Tilapia with higher FRS during the isolation had a higher probability to win the fight for social dominance, indicating dominance can be predicted using the FRS of the fish during isolation, provided that FRS values are not too close to each other. The dominant fish had substantially improved growth that was accompanied by higher expression of hepatic IGF-1 mRNA, a proxy of growth rate ($P < 0.05$). Based on this research, feeding responses of broodstock in isolation are good predictors of social status, such that individuals with low stress responsiveness (higher FRS or lower DAI) are likely to be dominant individuals that can can be selected for as broodstock. In a second study, we assessed the effect of broodfish behavioral stress response on seed production of tilapia through evaluation of their FRS during isolation. Social groups of broodstock representing low stress response (LSR) breeders predicted to be dominant individuals based on elevated FSR during initial period of isolation, or high stress response (HSR) breeders predicted to be subordinate individuals based on low FRS, and their combination were bred in hapas installed in ponds. The greatest number of eggs, largest egg size, highest sperm motility and sperm density, and most number of fingerlings produced were collected in treatments that had both LSR male and female breeders in the group. Higher rates of hatching and survival were also reflected in treatments that had either a LSR male or female in the group. The poorest values of seed quality and quantity were found in breeding groups that contain HSR males and females. These results indicate that stress responsiveness of

broodstock is a good predictor of fecundity and can be used to select fish with higher seed production. Use of feeding responses during an initial period of isolation can be used by hatchery operators and farmers to select broodstock individuals that produce higher quality and quantity of tilapia seed.

In a third, study we evaluated if fingerlings derived from LSR might have improved growth performance relative to fingerlings produced from HSR broodstock during a 3-month pond growout. Sex-reversed fingerlings derived from LSR breeders, had a higher average body weight, a better feed conversion ratio, and an overall higher yield per hectare than fingerlings derived from HSR breeders. There were no differences in survival among the two groups. The results suggests that fingerlings derived from LSR broodstock pose an advantage over those produced from HSR broodstock in overall production performance. This would likely be further amplified were fish growout extended to 4 or 5 months.

An additional study investigated the effect of stocking density on the growth, hepatic IGF-I, and stress responsiveness of fingerling tilapia reared in nursery hapas for 30 days. The overall effect of density as a stressor showed that low density, sex-reversed fish (250 fish/m^3) responded well in terms of growth, specific growth rate, survival, hematological profile (elevated red blood and white blood cell counts) and IGF-1 mRNA gene expressions compared to fish reared at higher densities (500 and 1000 fish/m^3). No clear pattern of difference in blood glucose levels was observed with stocking density. The research demonstrates that density-dependent stress impairs growth through inhibition of IGF-1 production. It is clear that IGF-1 mRNA is a strong growth rate indicator in the field and may also serve as an indirect measure of stress in tilapia. This along with certain hematological parameters may allow for assessment of environmental variables that limit stress and best promote growth in tilapia aquaculture. These data suggest that densities of 250 fish/m^3 are best for growth of fingerlings in nursery hapas.

INTRODUCTION

The quantity of Nile tilapia culture has risen significantly in the Philippines, by almost 4% annually, with a 33% increase between 1997 and 2002 (BFAR 2006 www.bfar.da.gov.ph). To support the growing tilapia industry, there is a need to provide year-round, high-quality seed that can be widely distributed at reasonable costs to tilapia farmers. This can be achieved through better broodstock quality, increased hatchery development and enhanced technologies for consistently high-quality seed production. Here, we aim to assess seed production efficiency in *O. niloticus* as a corollary of broodstock response to social stress. In the aquaculture and breeding environment, fish species such as the tilapia may develop various problems associated with physical, chemical and social stressors (Binurameeh et al. 2005; Chandroo et al. 2004), including impaired reproductive performance. Exposure to stressful conditions can reduce egg size and sperm count, cause ovarian resorption of eggs, delay ovulation, increase developmental abnormalities and reduce size and survival of offspring (Maeda and Tsukumura 2006). The effect of stress on fish is not only determined by the aversive character of the stressor but by the fish's cognitive appraisal of the stressor (Koolhaas et al. 1999). In order to optimize reproductive performance of valuable broodstock and improve seed production, stress must be limited and fish selected for based on their ability to better cope with stress. Breeding is largely driven by social behavior and an understanding or ability to predict dominance, or select breeders with reduced stress responsiveness, can improve hatchery and breeding programs.

The physiological and behavioral responses to stress may be used to select broodstock with reproductive advantage. The variable color pattern in fish may signal a behavioral strategy, enhance camouflage, improve communication, and confer reproductive advantage (Korzan et al. 2008). In *O. niloticus*, eye color is associated with social status (Volpato et al. 2003; Vera Cruz and Brown 2007). In our previous studies, eye color was found to be a predictor and consequence of social rank (Vera Cruz et al. 2009). However, no study to date has examined if eye color pattern is related to behavioral stress responses in *O. niloticus*; for instance, that associated with appetite inhibition or feeding response during isolation.

Moreover, to our knowledge, no study has been done in tilapia to examine if reproductive performance and seed quality is affected by broodstock social rank and/or condition.

To seek higher incomes, farmers in the Philippines are increasingly rearing fish at higher stocking densities in cages, concrete tanks and ponds, including more intensive fingerling production in nursery hapas. Filipino farmers are interested in knowing the stocking densities that yield the best growth rate and minimize mortalities under more intensive culture conditions. Some have noticed significant mortalities in cages, likely due to overcrowding. It is possible that the farmers are stressing, and therefore, reducing the growth potential of fish at higher densities. At lower densities behavioral or social hierarchies may dominate and limit growth potential. Therefore, an additional aim of this investigation will test the effects of stocking density on the growth, survival, and hepatic gene expression of IGF-I, a proxy for growth in *O. niloticus* and other fishes (Picha et al. 2008a), and on stress responsiveness in tilapia. Stress could be measured by a number of factors including survival, growth and elements of the growth axis (i.e. IGF-1), hematological variables (red and white blood cells), glucose, cortisol that is a key hormone mediating stress, and tissue indices (hepatosomatic and cholecystic index) (Bonga et al. 1997).

Physical and social stressors can evoke non-specific physiological responses in fish (Barton 2002). These responses are considered adaptive to enable the fish to cope with the stressful condition and maintain its homeostatic state. If the stressor is severe or long-lasting and the fish is not capable of regaining homeostasis, then the responses themselves may become maladaptive and threaten the fish's health and well being (Barton 2002). Physiological responses to stress can be grouped as primary, which include endocrine changes such as measurable levels of IGF-I (Vera Cruz and Brown 2007) and circulating catecholamines and cortisol (Barcellos et al. 1999) and secondary, which includes changes in features related to metabolism, immune function, and energy depletion reflective in lower liver weights ((Binuramesh et al. 2005; Picha et al. 2006; Picha et al. 2008b). Stressful condition was found to significantly increase circulating cortisol levels (Bolasina et al. 2006), hepatic phosphoenolpyruvate carboxykinase activity (Dibattista et al. 2006), and bile retention (Earley et al. 2004), but it significantly decreases hepatic IGF-I levels (Vera Cruz and Brown 2007). A well-characterized physiological consequence of social stress and excessively high densities is a reduced growth rate (Sloman et al. 2000). Excessively high stocking density is a stressful condition and decreases fish growth (Björnsson 1994), increases plasma cortisol levels in flounder (Bolasina et al. 2006) and decreases survival in salmonids (Sodebergg and Meade 1987). Social stress and the formation of feeding hierarchies, are also density dependent (Vera Cruz et al. 2006). Differential alterations in growth rate between dominants and subordinates are attributed more to behavioral changes (*i.e.* feeding) as transduced by physiological regulators (*i.e.* IGF-I level) but may also be due to changes in metabolism (*i.e.* hepatic phosphoenolpyruvate carboxykinase activity and bile retention) (Earley et al. 2004; Dibattista et al. 2006; Vera Cruz and Brown 2007). The growth-promoting actions of growth hormone (GH) are mediated through induction of IGF-1 (Degger et al. 2000; Picha et al. 2008a). Subordinate or stressed fish is characterized by larger somatostatin-containing neurons in the hypothalamus, which leads to reduced production of pituitary GH (Hofmann and Fernald 2000). Due to this, subordination depresses hepatic IGF-I levels while dominance stimulates its production, likely through greater secretion of pituitary growth hormone (Vera Cruz and Brown 2007). Here, we aim to assess densities that yield good growth and survival while simultaneously evaluating the use of IGF-I and various other factors as markers of growth and stress. An assessment of hepatic IGF-I mRNA in these studies will further validate its usage as a field indicator of growth status in tilapia (Vera Cruz et al., 2006; see Picha et al. 2008a) as well as its putative use as an indirect marker of stress. Additionally, blood glucose, red and white blood cell counts, and tissue indices (hepatosomatic and cholecystic) that can be readily measured will be evaluated as potential indicators of stress that could prove useful tools to evaluate poor environmental conditions. Building biotechnology capacity in the Philippines and development of potential bioindicators can expedite the evaluation of environmental parameters that best promote growth (or limit stress) in tilapia.

OBJECTIVES

1. Determine if physiological and/or behavioral responses to stress can be used in the selection of broodstock with reproductive advantage in Nile tilapia (*Oreochromis niloticus*).
2. Examine the effect of broodstock social condition on seed production and fingerling growout performance of tilapia.
3. Investigate the effect of stocking density on the growth, gene expression of hepatic insulin-like growth factor-I (IGF-I), and stress responsiveness of tilapia.

MATERIALS AND METHODS

Study 1 - The influence of duration of behavioral stress response on social dominance in tilapia

This study aims to investigate whether the outcomes of competition for social dominance among *O. niloticus* individuals can be predicted using the feeding response score and/or duration of appetite inhibition as a stress coping style. In addition, it also investigates if eye color pattern (ECP) is related to the duration of behavioral stress response such as appetite inhibition. The concept is to enable the selection of those broodstock that show dominance and hence might convey reproductive advantages. Physical and behavioral markers such as eye color pattern (ECP) and appetite, respectively, are features that could be easily assessed by hatchery operators in selecting the best mating pairs for seed production.

Phase 1 - Isolation and monitoring of the duration of appetite inhibition (DAI)

This phase of the study was done to assess if the outcomes of competition for social dominance might be predicted by DAI as a stress coping style.

Experimental fish

One hundred size #20 genetically male Nile tilapia *(Oreochromis niloticus),* with average weight of 0.60 g, were obtained from the Phil-Fishgen, Central Luzon State University, Science City of Muñoz, Philippines. They were maintained in a rectangular tank (2m x 1m x 1m) receiving continuous flow of water. The fish were fed three times a day at 3% of the body weight. Prior to isolation weight of each fish was determined.

Isolation and monitoring of the DAI

Fifty fish (mean weight of 26.02 ±0.98 g) were isolated at random in glass aquarium (30cm x 15cm x 30cm) for 10 days. Each isolation unit was aerated to ensure sufficient dissolved oxygen available for the fish. Three sides of the aquarium were covered to prevent the fish seeing other fish isolated in the nearby aquaria. Upon introduction of each fish in the isolation unit, it was immediately hand fed with three pieces of floating feeds placed in a feeding ring. The duration from the time of feed introduction to the time of feed consumption was regarded as the DAI. The DAI and the weight of the fish served as the bases for pairing the fish for social interaction; fish with shorter DAI against those with longer DAI; with both fish having similar weight. Fish were then fed daily at 1% of the body weight except two days prior to interaction. Water exchange was done every other day to maintain good water quality.

Social Interaction

After establishing the competing fish for social interaction, each fish in a pair was individually marked by a small cut on the upper or lower part of the tail fin for the purpose of identifying the fish with shorter and longer DAI. The fish in a pair with longer DAI was cut on the lower portion of the caudal fin and vice versa. After marking the fish, the pair was introduced into a new environment (30cm x 15cm x 30cm aquarium) at the same time to prevent the effect of place familiarity. The period from time of introduction to the time of first agonistic attack was recorded. The number of attacks in a ten minute-time from the first agonistic attack was separately recorded from the total number of attacks during the entire

interaction. Change in eye color pattern (ECP) of the competing fish was monitored at the start, during and after the competitive social interaction. Eye color was quantified as darken area of both iris and sclera (Volpato et al., 2003). The circular area of the eye was divided into eight equal parts using four imaginary lines (Figure 1). Eye color pattern value ranged from zero (no darkening) to eight (total darkening) . At the end of the interaction, social rank (dominant or subordinate) was identified by the characteristics displayed by each fish such as proactive and reactive, pursuing and retreating, erected and not erected dorsal fin and as well as changes in skin color and ECP. Canon power shot A650IS image stabilizer AIAF digital camera with resolution of 12.1 megapixels was used to document the social interaction which in turn was used in checking the observations made during the interaction.

Growth Rate Observation
Paired fish after the interaction were transferred to the dominant fish's aquarium to support its dominancy status. They were maintained for a week and fed once a day at 3% of their total body weight. Exchange of water was done every other day to maintain good water quality. The weight of fish was recorded a day after the fight.

Statistical Analyses
Frequency difference was analyzed using Binomial test. Mean DAIs of the two groups, and mean decrease in weight a day after the social encounter between dominant and subordinate fish were compared using paired sample T-test. Linear relationship of DAI and aggression was assessed using linear regression and Pearson correlation coefficient. Statistical analyses were carried out by using the SPSS software version 16.0.

Phase 2 - Feeding latency as an indicator of stress coping style
The second phase followed similar protocol as the first phase except we evaluated whether the outcomes of competition for social dominance among *O. niloticus* individuals can be predicted using the feeding latency score as a stress coping style. The feeding latency scores allow a quantitative evaluation of the feeding response exhibited throughout the whole isolation period.

Experimental Fish
The Freshwater Aquaculture Center (FAC) Selected Tilapia (FaST) strain of Nile tilapia fingerlings were obtained from the FaST project, FAC, CLSU, Science City of Muñoz, Nueva Ecija. Three hundred mixed sex tilapia of same age and similar sizes were reared in a rectangular tank (2 x 1 x 1 m) and fed two times a day at 3% body weight. Upon reaching 10 grams, male fish were separated from the females.

Feeding Latency During Isolation
After rearing in the rectangular tank, the fish were individually weighed and then isolated for 10 days in 30 x 30 x 15 cm aquarium. The fish were fed once a day at 1% of the body weight except during the last 2 days of the isolation period. The time from introduction to a new environment to the first acceptance of food was monitored for each of the fish. Feeding behavior during the entire isolation period was quantified by assigning corresponding point scores for a particular feeding behavior as shown below:

Point	Behavior
0	Fish does not react or eat the food
1	Fish eats only pellets directly put in front of it, and does not move to eat the food
2	Fish moves to eat the food, but comes back to its original place in the aquarium between each feeding
3	Fish eats all food present in the aquarium

After the isolation period, the daily scores were summed up to get the feeding response or latency score (FRS). Water exchange (50%) was done every other day to maintain good water quality. Water temperature was monitored daily at 7 AM and 2 PM.

Eye color was monitored daily during the isolation period. Eye darkening has been studied in other fish using gradual color patterns transformed into scores. Eye color was quantified as darkened area of both the iris and sclera as described above. Eye color pattern value ranged from zero (no darkening) to eight (total darkening).

Social Interaction

Fish with the same sex and similar weight but with different FRS were then subjected to social interaction. A day before initiation of the social interaction each fish in a pair was individually marked by a small cut in the upper or lower part of the tail fin. The location of the cut was dependent on the feeding latency score. The fish in a pair with higher feeding latency score was cut on the upper portion of the fin while in the other fish, the cut was on the lower portion of the fin. To prevent the effect of home location familiarity, the fish in a pair was introduced into a new environment (30 x 30 x 15 cm aquarium) at the same time and separated by a center divider. Body weights of dominant and subordinate individuals were recorded before and after the first encounter. Every morning, the division of the aquarium was removed for ten minutes and the social interaction of the fish was observed. After daily interaction, both fish were then fed to satiation once a day and after 14 days of pairing, the social status (behavior, eyes and body color) of each fish was measured.

Growth Rate Observation and IGF-1 mRNA Analyses

The weight of each fish was recorded weekly over the 14-day social interaction period to establish changes in growth among the dominant and subordinate fish. At the end of the study, ten pairs of fish were sampled for liver for extraction and determination of IGF-1 mRNA levels.

Total RNA from the liver was purified using Trizol® (Invitrogen™, Carlsbad, California, USA) using the protocol suggested by the manufacturer. The purity of the isolated samples was assessed by using the A260 : A280 ratio which ranged from 1.6-2.0, with most reading ranging from 1.9-2.0. The amount of RNA in ng per µl sample served as the basis for the addition of 1 µg total RNA template in the reactions. First strand cDNA was generated in 20 µl RT reactions with 1 µg total RNA template, Omniscript® reverse transcriptase, 10X RT buffer, 5 µM dNTP, 10 µM oligo-dT primer (Promega®, Madison, Wisconsin, USA), and RNase inhibitor (RNasin®, Promega®). Samples were reverse transcribed by incubation at 37 °C for 60 min. The IGF-I mRNA was quantified using the TaqMan real time quantitative reverse transcriptase – polymerase chain reaction (qRT-PCR) assay described in Vera Cruz et al. (2006) which was performed on Lightcycler® 480 II (Roche Ltd., Basel, Switzerland). Values for IGF-1 mRNA were normalized to total RNA.

Statistical Analyses

Frequency difference was analyzed using Binomial test. Linear relationships of feeding latency percentage, aggression, ECPs and durations of social interaction were assessed using Pearson correlati coefficient. Durations of appetite inhibition and interaction was analyzed using Chi square test. Statistical analyses was carried out using the Statistical Analysis Software (SAS).

Study 2 – Effect of Broodfish Social Condition on Seed Production of Nile Tilapia

This experiment was undertaken to assess the effect of broodfish behavioral stress response (BSR) on seed production of *O. niloticus* through evaluation of their feeding response score during isolation.

Determination of Social Groupings
Two potential social groupings in relation to broodfish behavioral stress response (BSR) of tilapia breeders during isolation were used in this study: the low stress response (LSR) and the high stress response (HSR) groups. The LSR breeders were those that manifested shorter period of adjustment in response to stress experienced after transfer to a new environment as indicated by a higher feeding response score (FRS). While the HSR breeders on the other hand, were those that exhibited longer period of coping with stress or those that had lower FRS. Their BSR were quantified through feeding response evaluation during the isolation period

Male and female FAC selected Nile tilapia (FaST) breeders of the same ages with body weights ranging from 75 – 100 grams were used in the study. The stocks were acquired from the FAC, CLSU, Science City of Muñoz, Nueva Ecija. The breeders were stocked for 10 days in rectangular tanks (2.30 x 1.30 x 1 m) prior to isolation. A total of 30 male and 90 female FaST breeders were used to represent all the social groups in all the treatments.

Glass aquaria measuring 30 x 60 x 30 cm were used as isolation chambers for seven days for the randomly selected male and female FaST. Each unit was provided with aeration system to sufficiently provide the dissolved oxygen requirement of the fish. Three sides of each aquarium were covered by a white plastic to avoid seeing other fish in the adjacent isolation chambers. Prior to isolation, weight of individual fish was recorded. Along with the BSR through feeding observations, the weights obtained were used as basis in the distribution of the breeders in their respective treatment assignments. This parameter was also considered in the group allocations to prevent weight from being a factor in the reproductive performances of the breeders and ensure that the results obtained were only affected primarily by their BSR.

Once fish were assigned to their respective isolation chambers/aquaria, they were hand fed once a day with a commercial diet at a rate of 1% body weight. Feeding rings were used when feeds were administered to be able to clearly observe the possible feeding response that the fish might manifest. The time the fish resumed feeding after the transfer to the new environment and the particular feeding response executed until the 7^{th} day of the isolation period were closely monitored and recorded. After the isolation period, points were summed up to get the total feeding response score (FRS). Breeders that accumulated feeding points ranging from 9 to 17 were assigned to the low stress response (LSR) group, while breeders with points ranging from 0 to 4 belonged to the high stress response (HSR) group. Since weight was also considered, the fish which demonstrated good feeding response but had higher size variations were rejected.

Feeding behavioral response manifested during the entire isolation period was quantified through corresponding points assigned for a particular feeding response as indicated previously. The scoring is as follows:

0 points - fish does not react or eat the food within a 2 minute time frame;
1 point - fish eats only pellets directly put in front of it, and does not move to eat the food other feeds;
2 points - fish moves to eat the food, but comes back to its original place in the aquarium between each feeding; and
3 points - fish eats all food present in the aquarium regardless of the position of feed pellets within the aquarium

Seed Production Evaluation
The feeding response score (FRS) obtained and breeders' weights were the bases for the social groupings. The selected breeders were distributed in the two social groupings; LSR and HSR groups, where each group was composed of 15 male and 45 female breeders. The sex ratio was one male: three females. The

breeding was done in hapas (1 x 2 x 1 m) installed in ponds with a stocking density of 4 pcs/m^2, which is typically used for breeding tilapia.

The description of the assignment of fish in their respective 5 treatment grouping is as follows:
LSRHSR♂♀ = 1 HSR male, 1 LSR male, 3 HSR females, and 3 LSR females,
LSR♂♀ = 2 LSR males and 6 LSR females,
HSR♂♀ = 2 HSR males and 6 HSR females,
LSR♂HSR♀ = 2 LSR males and 6 HSR females, and
HSR♂LSR♀ = 2 HSR males and 6 LSR females

Each treatment was replicated three times.

After the 14 days breeding period, female breeders were inspected for the presence of eggs and fry. Eggs were collected from the mouth of the female breeders by pressing the mouth of the fish and gently opening their operculum through the collector's thumb and index finger. In case of existence of swim-up fry, collection was done using scoop nets. The swim-up fry collected were temporarily placed in a plastic basin with water and immediately counted.

The parameters observed and recorded include spawning success, total seed produced, hatching rate and survival rate. The egg quality in terms of size through its diameter was also evaluated. As for the male breeders, sperm analyses through motility scoring and sperm density were also observed.

Egg Quality: From each replicate, 25 eggs were randomly selected to represent the whole population. Since the eggs were oval in shape, the diameter of each egg was carefully measured at their longest portion using a vernier caliper.

Sperm Quality: The procedure done by Danting (1992) for scoring sperm motility was followed. Sperm motility in all samples was scored on a subjective rating scale system of 0 to 10. A rating of 10 denotes that 100% of the spermatozoa under observation are motile, moving actively, while zero (0) rating indicates that no sperms are moving after activation. Prior to scoring 20 µl of sample was diluted in 100 µl water for activation. Following activation, sperm motility scoring was determined under the microscope. Only sperm swimming in a forward motion were included in estimation of motility.

Sperm density was estimated using a Neubauer slide counter (Haemacytometer, 0.1 mm., 1/400 mm^2, Weber Scientific, England). Before the milt was used for any purpose, sperm head counts were done to estimate the whole sperm sample densities. Milt was subsequently diluted and a 10 ul was dropped on the Neubauer Slide (Haemacytometer) for counting. The slide was left for approximately 10 minutes to allow the sperms to settle into one plane.

Spawning Success: The average number of eggs produced was evaluated on the 2nd production cycle, since the first cycle already contained swim-up fry. Average egg production per female was calculated as the number of eggs collected / number of female breeders that spawned. Spawning success was determined by percentage spawning rate [(number of female breeders/total number of stocked female breeders) x 100]

Hatching Rate: During the incubation period, a rate of water flow was maintained to allow a continuous movement of the eggs which also prevents their damage, clumping and settlement. Round bottom incubation jars were used, since previous studies have proven that these containers can give good results provided that water flow rates and water quality are carefully regulated (Subasinghe and Sommerville, 1992). Percent hatching rate was calculated as number of hatched eggs/total number of eggs x 100.

Fry Survival Rate and Size: When the yolk sac had been absorbed, the fry were transferred in the nursing hapas installed beside the breeding and conditioning hapas. Stocking was done in the morning. The fry were reared for three weeks or until size # 24 was obtained. Daily feeding (twice a day) of commercially available fry mash at five per cent of their body weights was done to optimize their growth. After the rearing period of three weeks, the post-fry were collected and their survivorship determined (number of post fry survived/total number of fry x 100).

The harvested post-fry for both production cycles were subjected to length and weight measurements. For the determination of their lengths, 20 samples were randomly selected for each replicate in each treatment, in each batch. Each post-fry was measured using a vernier caliper. Weight was determined through dividing the acquired collective weight of sampled post fry by the number of individual fry.

Statistical Analysis

The treatments were determined in terms of the ratio of male to female, either singly or in combination, based on their identified potential social status. The treatments were allocated in each hapa following the Randomized Complete Block Design (RCBD) with three replications per treatment. Two-way analysis of variance for RCBD was done and Least Significant Difference was used to identify the effect of behavioral stress response of the fish among the social groupings. Appropriate analysis like analysis for unequal replications and data transformation were calculated using the General Linear Model via the SAS V9.0 software.

Study 3 – Effect of Broodstock Social Condition on the Growout Performance of Nile Tilapia Fingerlings

There were two phases in this study, the first phase was the determination of the social condition of the broodfish by isolation for 5 days in aquarium and observing feeding response. The feeding response was done once a day for the entire 5 days of isolation and quantified through the corresponding points assigned for a particular feeding response, as previously described above. The breeders that obtained total points ranging from 9 to 15 were assigned to the low stress response (LSR) group while those that had 0 to 8 were considered the high stress response (HSR) group. After determining the broodfish with LSR and HSR, males and female breeders of the same social condition were conditioned separately in conditioning hapas prior to the breeding period. After the breeding period, fry were collected after 15 days and were stocked for sex-reversal treatment in hapas for 21 days. Sex-reversed fingerlings were used for the second phase of the study – the grow-out period.

The second phase of the study was composed of two treatments that were replicated three times. Treatments were as follows: I – sex-reversed fingerlings produced from LSR broodfish, and II - sex-reversed fingerlings produced from HSR broodfish. Size 22 (ave. wt. ranged from 0.192 to 0.208 g) of sex-reversed Nile tilapia fingerlings were used in the study. Fingerlings were stocked in six 200 m^2 earthen ponds at 2 fish· m^{-2}. The fingerlings were fed twice a day with commercial feeds at 20% of the body weight from 0-2 weeks, 10% of the body weight from 2-4 weeks, 7% of the body weight from 4-6 weeks, 6% from 6-8 weeks, 5% from 8-10 weeks, 4 % from 10-12 weeks.

Regular fertilization was made using inorganic fertilizers such as ammonium and 5.6 kg P·$ha^{-1}week^{-1}$ to enhance the growth of natural foods in pond water. Fertilization of ponds was dependent on the productivity of the pond water. Secchi disc visibility readings were maintained at 40 cm and below.

Eighty individuals were sampled biweekly to obtain average weight and length using cast net method as a sampling device to check the growth of stocks. At the end of the culture period, 80 fish or 10% of the total fish stock were sampled for individual weight and length.

Water quality parameters such as dissolved oxygen, water temperature, pH, total ammonia-nitrogen; alkalinity and phosphorus levels were monitored weekly starting at 9 AM in the morning.

After 90 days of culture period, all data were gathered and analyzed by T-Test in randomized complete block design with three replications.

Study 4: Effect of Stocking Density on the Growth Responsiveness, and IGF-1 Expression in Nile Tilapia

Along with rapid expansion and intensification, there is a growing concern on the welfare and health of farmed fish. Vahdatpour et al. (2009) and Mostl and Palme (2002) pointed that stocking densities in commercial aquaculture have been highlighted as a subject of increasing importance as far as fish health is concerned. Any alteration in the physical and psychological state of a living organism as it interacts and responds to environmental variations (Chandroo et al., 2004) like rearing at higher stocking densities induce stress. With aquaculture's expansion, more accurate information on stress control is highly necessary in order to assure health of fish. Scientific investigations have shown interest on early detection of stress in fishes that has led to increased study of potential biochemical, subcellular, cellular, histological and behavioral markers or biomarkers of stress.

This experiment assesses the effects of stocking density on *O. niloticus* growth and survival, hepatic IGF-I gene expression and stress responsivenss associated with stocking densities. We initially intended on measuring cortisol as a marker of stress in this study, but the plate reader intended for its measurment at CLSU was in disrepair and required substantial modification to measure plasma cortisol by an ELISA validated by our group (Cayman, Ann Arbor MI. USA). An alternative approach would be to measure cortisol by a radioimmunoassay (Dean et al. 2003). However, this technique requires isotopes not permitted for use at CLSU. Also, because of substantial time required for shipment and delicate nature of the samples they could not be reliably sent to NCSU for measurement. Nevertheless, we modified our objective to measure not only survival, growth and hepatic IGF-1 gene expression, but various other possible stress sensitive parameters including hematological, glucose, hepatosomatic and cholecystic indices. As good management provides the key to the avoidance of essentially all health problems whether stress related or not measurement of these variables will both establish reference ranges for Nile tilapia and those potentially linked to stress that could be used to assess overall health status of fish.

Rearing and Conditioning of Fingerlings in Net Enclosures

Seven (7) conditioning enclosures "hapas" with measurements of 2 x 5 x 1 m (10 m^3) were installed in a 1000 m^2 pond at the FAC-CLSU. Stocking density for each conditioning hapa was 150 pieces/m^2 or 1,500 pieces/hapa. The first two nets were stocked with mixed sex tilapia and the remaining five nets were stocked with sex-reversed fish. Fry mash was given twice daily, with a feeding rate of 20% of the fish biomass on the first week, and adjusted to 11% on the second week. On the third week, the feeding rate was lowered to 10% of the fish biomass and on the last week of rearing, the feeding rate was lowered to 6.5%. A support set of fingerlings were also reared and conditioned in 200 m^2 pond at the Genetic Improvement of Farmed Tilapias-FeedMix Fortified ($GIFT_{FF}$) facility at CLSU. The stock included 20,000 pieces of size 20 fingerlings with an average weight of 0.6 grams. Fingerlings were fed 15% biomass on the first week, 13 % on the second week, 10% on its third week and 9% on the last week. Growth rate was monitored weekly.

After acclimatization, 8,000 experimental fishes were distributed randomly in four treatment groups and each treatment was replicated four times. Fish were fed 2% of the biomass. All treatments were set in 16 experimental units following a Completely Randomized Design (CRD). The treatments used were as follows:

T1 - 250 fish/m^3, control, low density, mixed-sex
T2 - 250 fish/m^3, low density, sex-reversed males
T3 - 500 fish/m^3, medium density, sex-reversed males
T4 - 1000 fish/m^3, high density, sex-reversed males

Fish weights were obtained from fish at the beginning and end of the experiment. SGR was also determined over the course of the experiment by obtaining fish weights at 10 (March 19), 24 (April 2), and 30 days (April 8 – end of experiment)

Water Quality Monitoring: The temperature ($^{\circ}$C) and dissolved oxygen (mg/li) were monitored in the morning and in the afternoon during the 30-day nursery period. Water transparency was determined using a Secchi disk. The average Secchi disk readings were used in the calculation of Trophic State Index (TSI = 60-(14.41*[Ln (average Secchi disk readings)]).

Hepatic IGF-1 mRNA: Fingerlings from net enclosures and ponds were drawn for the initial, basal measurement of hepatic IGF-1 mRNA. These samples represented the following: three females and three males from mixed-sex fingerlings reared in hapa, three males from sex-reversed fingerlings reared in hapa, and three males sex-reversed fingerlings reared in ponds.

For the experiment, a total of 48 juvenile fish samples were collected for IGF-1 determinations from each replicate at 7, 15 and 30 days (Mar 16, Mar 24 and Apr 8) from initiation of the experiment. The weight of each fish sample was taken and recorded. An incision started off at the anal region up to the abdominal part to expose the liver of the fish. Hepatic tissue samples were collected and immediately placed in 0.5 ml microcentrifuge tubes and frozen in liquid nitrogen.

Total RNA was isolated from the hepatic tissue using Trizol (Invitrogen™, Carlsbad, California, USA). Glycogen in the samples were removed using a high salt solution. Two reaction cycles were done on RNA samples to remove possible genomic DNA contamination using DNA-free™ (Ambion, Austin, Texas, USA). The RNA concentration was quantified by spectrophotometry at 260 nm and its purity was checked by obtaining the 260/280 ratio which ranged from 1.90 to 2.05 (NanoDrop spectrophotometer, MSI U 100 Series, Wilmington, DE, USA).

Sample RNA was reverse transcribed to produce cDNA. This cDNA was subsequently measured by TaqMan® real time quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) as previously described (Vera Cruz et al. 2006). A serial dilution of cDNA was run to generate a standard curve (the log of initial target copy number was plotted against the threshold cycle) of IGF-1. The amount of IGF-1 mRNA in each sample was calculated by substituting the generated threshold cycle values to the equation derived from the standard curve (Bustin 2002). IGF-1 mRNA (ng cDNA) was normalized to sample total RNA (ul total RNA).

Hepatosomatic and Cholecystic Indices: Samples of the fish's liver and gall bladder were weighed and recorded from each replicate after the 30-day nursery period. The hepatic tissue and gall bladder were collected after dissection then weighed and measured using an electronic scale (SANFORD 1261552, 0.01g; 2000 capacity). The hepatosomatic (liver weight/body weight x 100) and cholecystic (gall bladder weight/liver weight x 100) indices were measured

Hematological and Blood Glucose Profiles**:** Blood samples from each replicate were drawn by cardiac puncture using 1cc tuberculin heparinized syringe. Blood sample from each replicate was pooled in heparinized 1.5 ml tubes and placed temporarily in crushed ice. Whole blood glucose was determined using a glucose meter (Glucostar-Glucometer). Total red blood and white blood cells (WBC) were

counted using- Hemacytometer ((Neubauer,USA). The Turk solution for WBC counting was prepared using 0.05 g gentian violet, 1 ml glacial acetic acid and 100 ml distilled water. The Gower solution for RBC counting was prepared by mixing 9.38 g sodium sulfite anhydrous, 25 ml glacial acetic acid and 150 ml distilled water.

Rearing of Fingerlings in Tanks
Parallel investigations were initiated in semi-intensive tanks at the NCSU Pamilco Aquaculture Field Station (Aurora, NC). Sex-reversed Nile tilapia for this study were stocked in 1500 L tanks at a density 5, 10 and 30 kg m^{-3}. Following a 3-week growth period, the experiment was terminated by hurricane Irene in August 2011. PAFL was highly damaged and lost its dormitory and emergency backup generator from massive flooding. The fish from the experiment did not survive.

Statistical Analyses
Data on hepatic IGF-1 mRNA, growth, hepatosomal, cholecysctic, glucose and hematological indices from each replicate of each treatment are expressed as mean ± standard deviation. All data were analyzed statistically by General Linear Models procedure (SAS 1999). Tukey's Multiple Range (5% probability level) was used to test the differences between treatment means. A paired comparison using Proc Means of the Statistical Analysis System (SAS) was also done on variable weight, survival and IGF-1 to test whether there is a significant difference prior to and post stress evaluation of fish reared in different densities.

RESULTS AND DISCUSSION

Study 1 - The influence of duration of behavioral stress response on social dominance in tilapia

Phase 1 - Isolation and monitoring of the duration of appetite inhibition (DAI)
DAI after transfer to isolation units:
The mean DAI for all isolated fish was 83.55 ± 14.29 (mean ± SEM) minutes. The shortest DAI was 0.31 minute and the longest was 570.76 minutes. After the matching pairs for later pairing had been established, short DAI group had a mean DAI of 33.55 ± 10.15 minutes, which was significantly shorter ($P < 0.01$) than that of the long DAI group (133.54 ± 22.86 minutes; Figure 2).

Social interaction:
During the introduction of competing individuals in the aquarium, both fish displayed pale body coloration with dark stripes. The mean duration before observance of first attack was 10.86 ± 2.13 minutes. The fastest individual to adapt to the social condition and that attacked the opponent took less than 6.0 seconds, while the longest duration before observance of first attack was 33.66 minutes. However, at the beginning of the social encounter it was not always the fish with shorter DAI that initiated the fight. Thirteen (52%) of the 25 fish with shorter DAI (compared to their respective opponents) initiated the fight, while fish with a longer DAI initiated 11 social interactions. One pair did not show any interaction.

During the social encounter, the dorsal fins of both fish were raised and both swam towards each other indicating their preparedness to fight. Then they began aggressive interaction which involved chasing, rapid circling and biting directed against the mouth, fins and other body parts of the opponent. During this period of intensive interaction, both fish exhibited pale body stripe coloration. However, during the later part of the interaction, challenged fish mostly rebuffed attacks and eventually one of the fish chased and bit the flanks of the other fish that was fleeing. At this point, aggressive behaviour became unidirectional, and an aggressive dominant individual and a retreating subordinate fish was clearly identified. It was also observed that body- and eye-darkening of the fish increases with subordination and declines with dominance.

Overall, formation of a stable dominant-subordinate relationship was observed in 24 out of the 25 tested pairs for social dominance. Seventeen dominant fish (70.83%) of the 24 had shorter DAI during isolation compared to their opponents. This frequency difference on DAI of the dominant individuals was significant (Binomial test, $P = 0.03$; Figure 3). However, as previously mentioned, social encounter was not always initiated by the earlier eaters (i.e. shorter DAI), but eleven (64.70%) of the 17 dominant earlier eaters initiated the fight and the remaining six individuals did not start up attacking the opponent yet won the fight. On the other hand, five later eaters that became dominant began the fight, while the remaining two did not.

The recorded mean number of attacks of the 24 pairs before winning the fight was 73.33 ± 14.31. The most aggressive pair had 201 attacks in a 10-minute fight and had 277 attacks in the whole course of interaction. By contrast, the least aggressive pair made no more than one attack before the establishment of dominance. The DAI difference and level of aggression (number of attacks) between the competing pairs showed an insignificant weak positive correlation ($r = 0.28$, $P = 0.193$).

Dominance/Subordination and Growth

Reduced growth rate is a well-described physiological end result of social stress. The mean weight of subordinate fish before the interaction was 26.17 ± 1.40 g and this was reduced to 23.29 ± 1.36 g one day after the fight. While in the dominant fish, average weight was decreased from 26.81 ± 1.45 g to 24.70 ± 1.36 g (Figure 4). The mean decrease in weight was significantly higher for subordinate fish (2.88 ± 0.21 g) compared with dominant fish (2.11 ± 0.19 g) ($P < 0.01$).

Mortality of Subordinates

Death can be the most overwhelming effect of stress. After the interaction of each pair, winner and loser individuals were easily identified by their displayed behaviors. One day after the fight, one subordinate fish immediately died followed by four on the second day, nine on the third day, which was the day with the highest number of mortality. Another three died on the fourth day, five on the fifth day and one on the sixth day. The last surviving subordinate individual died on the seventh day after the interaction. It took one week from the day after the social interaction for all subordinate fish to die.

Phase 2 - Feeding latency as an indicator of stress coping style

Feeding Response Score and Social Interaction

We found that tilapia with higher FRS during the isolation had a greater possibility to win the fight for social dominance. Formation of stable dominant-subordinate relationship was observed in 59 of the 60 pairs tested. Thirty six fish of the 59 after the first interaction and 34 of the 56 after 14 days that became dominant had higher FRS compared to that of their conspecifics (Binomial test, $P < 0.05$). This indicates that social dominance can be predicted using the FRS of the fish during isolation so long as FRS values are not very close among the pairings.

Dominance/Subordination in Relation to Growth Rate and Hepatic IGF-1 mRNA Expression

Table 1 shows a summary of time before first acceptance of food and feeding latency or response score during the isolation, and weight of competing individuals before the interaction and 1, 7 and 14 days after the first interaction. Before the social interaction there was no significant difference on the body weight between the competing individuals (see Table 1). During and after the interaction, most of the dominant individuals have higher body weight than that of the opponent.

On growth of the fish, dominant fish had a mean SGR of 1.61% which was significantly higher ($P = 0.013$) than that of the subordinate fish (0.93%). The correlation of IGF-I mRNA and the specific growth rate (SGR) of the fish from day 7 to 14 of the social interaction period is shown in Figure 5. Generally, fish with faster growth rate had a higher IGF-I mRNA gene expression. The mean relative abundance of

hepatic IGF-I mRNA of dominant fish was elevated 73% when compared with the subordinate fish (P = 0.005) after 14 days. There was a significant overall positive correlation (r = 0.65) between SGR and relative abundance of IGF-I mRNA (P = 0.002).

Behavioral stress response can be used to predict outcome of contest for social dominance. Results of the present study indicate that tilapia with shorter DAI after its transfer to isolation in a new environment has a greater chance to become dominant when paired with another individual. The results that fish with low behavioral stress response became dominant in majority of the social pairing are similar to those established for the anole and rainbow trout (Korzan et al. 2006; Øverli et al. 2004; Pottinger and Carrick 2001). The time variation of resumption of food intake (ranging from seconds to hours) of fish after being transferred to new environment most likely reflects some aspects of the physiological stress responses to confinement, which could also affect the outcome of the social interaction (Øverli et al., 2004). According to Bernier (2006), stress induced inhibition of food intake in fish may, in part, be mediated by corticotrophin-releasing factor system which plays a key role in controlling the neuroendocrine, autonomic, immune, and behavioural responses to stressors. On the other hand, the fish's resumption of feeding after having coped with a stressful condition may reflect a down regulation of the physiological stress response (Øverli et al. 1998).

The results that not all fish with shorter DAI won the fight calls for a need to refine the method of assessing the behavioral stress response in this species of fish. In a review, Øverli et al. (2007) described how feeding behavior can be used as an indicator of stress coping style. Feeding behaviour can be assessed using point system based on the feeding behavior of the fish when fed daily for one week during isolation. In phase II of this study, we evaluated the FRS over a 10-day isolation period and found that social dominance can be predicted from animals that exhibit a higher FRS so long as FRS values among pairings clearly differ and are not too close to each other.

Social encounter is potentially costly and risky to the fighting opponents. The cost of fighting includes energy, time and physical injuries. The individuals engaged in social fight are integrating the costs and benefits associated with the contest and adjust their behavior accordingly (Hsu et al. 2006). At a certain point when an individual in a pair reaches its own dangerous threshold, an established dominant-subordinate relationship will be observed after one of the fish retreats or surrenders. In the current study, the observed changes in behavior and body and eye color of the competing fish served as social signals to the opponents to limit aggressive interaction. When social hierarchy had been established, subordinates showed increased body stripes and eye-darkening patterns relative to dominant fish which showed decreases in these variables. These observations conform with previous work (Volpato et al. 2003; Bero 2008; Vera Cruz and Brown 2007).
Social aggression is stressful for both dominant and subordinate fish (Summers and Winberg 2006). In social interaction, defeat in many animal species is a powerful stressor that can lead to drastic alterations in physiology and behavior. Behavioral effects of defeat include appetite inhibition (Gómez-Laplaza and Morgan 2003; Øverli et al. 1998; Winberg et al. 1993), reduced aggression (Höglund et al. 2001; Blanchard et al. 1995), and increased submissive and defensive behaviors towards conspecifics (Blanchard et al. 1993; Siegfried et al. 1984). The observed weight reduction in the current study after the interaction in both the dominant and subordinate fish supports our previous findings (Vera Cruz and Brown 2007). The reduced weight of subordinate fish a day after the social interaction may be more a result of appetite inhibition rather than a reflection of mobilization of stored energy for physical activity associated with social stress encountered. The subordinate fish were observed not consuming food after the social interaction and dominant fish even guarded or monopolized the food against the opponent. On the other hand, the increased physical activity of dominant fish during and after the aggressive encounters, a behaviour indicating that they have won the contest, may have contributed to the lower mean weight of the fish after the interaction. However, during the establishment of social hierarchy, the two social groups experienced similar level of physical activity. Thus, body weight differences between

the two social groups during the establishment of social hierarchy were mainly attributed to physiological and behavioral differences such as appetite rather than to differences in physical activities (Fox et al. 1997; Øverli et al. 1999). Inhibited food intake in subordinate fish may be due to social stress-induced increase in the serotonergic activity in the brain (Winberg et al. 1992) and/or neuropeptide Y mRNA expression in the preoptic area (Doyon et al. 2003).

The mortality of subordinates in Phase I of the studies is most likely a result of exhaustion caused by social stress. This was also observed by Petrauskiené (1996) in rainbow trout reared at low densities (2 or 3 individuals) where most of the subordinate fish may have reached the exhausted state during the third day. Subordinate fish confined with a dominant fish experience social stress and showed increased standard metabolic rate or a metabolic disadvantage (Sloman et al. 2000) that may lead to impaired growth. Our previous work shows that lower social status depresses hepatic insulin-like growth factor-I (IGF-I) levels while dominant status stimulates IGF-I production (Vera Cruz and Brown 2007). In Phase II of the present study we found that dominant fish predicted by an elevated FRS, exhibited increased growth and that this is associated with enhanced hepatic IGF-1 mRNA levels. With social stress seen with subordinates, hepatic IGF-I declines leading to reduced growth rate.

Overall, the results of these studies suggest that DAI or FRS are good predictors of social status in Nile tilapia with shorter DAI and higher FRS values during isolation leading to a significantly greater proportion of individuals that show dominance during social interaction. The dominant individuals have improved survival, enhanced hepatic IGF-1 gene expression and increased growth rate. The opposite occurs in subordinate tilapia whose social status and reduced capacity to cope with stress can be predicted from lower feeding response scores and a longer duration of appetite inhibition during a previous period of isolation.

Study 2 – Effect of Broodfish Social Condition on Seed Production of Nile Tilapia

Egg Quality: The measured average size of the eggs collected from each treatment is presented in Table 2. Eggs from LSR♂♀ (2.86 mm) were significantly bigger than those of the other treatments. Comparison of mean egg sizes in LSRHSR♂♀ and HSR♂LSR♀ revealed no significant difference but their egg sizes were significantly higher than those in HSR♂♀ and LSR♂HSR♀, which were comparable with each other.

The average size of eggs in this study ranged from 2.16 to 2.86 which is in agreement with the findings of Payne and Collimson (1983) who showed that eggs of *O. niloticus* usually range in size from 1.94 to 2.95 mm. Although mean egg sizes in all the treatments fell under the said range, the social grouping where both the breeders manifested LSR had a significantly higher egg size than the others. Rainbow trout (*O. mykiss*) subjected to a milder stress regime during early vitellogenesis produced smaller eggs that varied in size, while there was no effect on mean egg size in fish stressed during late vitellogenesis (Contreras-Sanchez et al. 1998). It is possible therefore that stress encountered by the HSR female breeders during vitellogenesis led to the production of smaller eggs in the LSR♂HSR♀ and HSR♂♀ treatments. Also during the whole isolation period, the majority of HSR breeders did not show any aggression towards the introduced feed and did not even eat the feeds within the two-minute time frame given. These behaviors by the HSR breeders might have also been manifested during the breeding period whereby a deficiency in nutrients may have contributed to the smaller egg sizes for these social groups.

Sperm Quality: The accumulated FRS of the male breeders with their corresponding motility scores are presented in Table 3. Sixty-five males were subjected to isolation for seven days, however, only 20 of them produced sperm during the collection. Twelve males were chosen to represent the respective FRS. Eighteen was the highest FRS a male breeder was able to accumulate during the whole isolation period, while, zero was the lowest gathered FRS.

The highest motility score obtained was 10 and the lowest was 3. The samples with accumulated FRS from 6 to 10 all attained a sperm motility score of 10. However, although they all had100% motile sperms, a difference in terms of active movement was also observed under the microscope. The male breeder with a 10 FRS had 100% motility, but only 75% of them were actively swimming and the remaining 25% showed a more moderate pace of motility. While the sperm collected in the male breeders with an FRS of 6-8 consisted of 100% motile sperms, only 50% of them were active and the other half showed slow motility. The breeders with an 11 to 18 FRS had 100% motility that consisted of very active and fast swimming spermatazoa. Low sperm motility could actually reduce fertility even despite an increase in sperm quantity (Kurokura and Oo 2008). Breeders with FRS of 0-5 showed relatively low motility scores, whereby 50% of their moving sperms were actively swimming and the other half showed moderate to slow movement. The breeder that produced the lowest motility score of 3 (FRS of 0), showed only 30% motility that was of moderate pace.

Table 4 presents the sperm density for the accumulated FRS by each male breeder. The accumulated feeding score from zero to six were considered to belong to the HSR group while those that accumulated 10 points and above were under the LSR group. Breeders that gathered 15 and 18 FRS obtained the highest sperm densities of 1.063×10^{10} and 9.400×10^{9}, respectively. On the other hand, zero and one FRS had the lowest densities of 2.025×10^{9} and 3.100×10^{9}, respectively. Male breeders that were able to accumulate 18 and 15 points had the significantly higher sperm densities than those of the other breeders. While, the breeder with FRS of zero had significantly lower sperm density compared to those of the other breeders with higher FRS. The trend shows that the FRS was directly proportional to the sperm density; the higher the accumulated FRS of a male breeder, the more sperm it produces. In the present study, LSR breeders show dominance over the fish that belong to the HSR group and show higher sperm density and motility. Hence, dominance may increase the sexual maturation rate in Nile tilapia males (Goncalves-de-Freitas 1999). In the African cichlid, *Astatotilapia burtoni*, social status determines reproductive capacity of males via increased activity of the of the brain-pituitary-gonad (BPG) axis, which leads to increased production of sex steroid hormones (Parikh et al. 2006; Burmeister et al. 2007). In the hypothalamus, gonadotropin releasing hormone (GnRH) stimulates the secretion of gonadotropins which stimulate male reproductive functions and secretion of testosterone hormone required for spermatogenesis and sperm transport (Bearden and Fauquay 1980). In bulls, previous studies revealed that GnRH treatment increases sperm concentration and levels of live spermatozoa (El-Azab et al. 1996; Gabor et al. 1998). Considering dominance increases the activity of the BPG axis in cichlids, which leads to enhanced production of gonadotropins, it is quite plausible that breeders with increased FRS and that manifest low stress response behaviours (show dominance) are the ones that show increased sperm density and motility.

Spawning Success: Table 5 presents the average spawning success per treatment during the 2nd production cycle while Table 6 presents the average number of eggs a female was able to produce in one spawning. Spawning success in LSRHSR♂♀, LSR♂♀, LSR♂HSR♀ and HSR♂LSR♀ were comparable and significantly higher than that in the HSR♂♀ group. The average number of eggs produced per female in the LSR♂♀ group was significantly higher than those in LSRHSR♂♀, LSR♂HSR♀ and HSR♂♀ but comparable with that in HSR♂LSR♀. The HSR♂LSR♀ group revealed no significant difference with that of LSRHSR♂♀, but was significantly higher compared to the LSR♂HSR♀ and HSR♂♀ groups. The LSRHSR♂♀ and LSR♂HSR♀ on the other hand were comparable. Average number of eggs produced in HSR♂♀ was significantly lower than those of the other treatments.

All treatments with both or either male or female LSR breeders (*i.e.* LSRHSR♂♀, LSR♂♀, LSR♂HSR♀ and HSR♂LSR♀) had comparable spawning rates that were significantly higher ($P < 0.05$) than those in the HSR♂♀, which was composed of HSR male and female breeders. However, the advantage of treatments that consisted of LSR female breeders (LSR♂♀, 951.67 ± 151.28 and HSR♂LSR♀, 797.00 ±

129.26) was that they produced more eggs per female per spawning than those in the groups with HSR female breeders ($P < 0.05$). Interestingly they also had increased egg size (Table 2).

Eggs and Fry Production: The total number of eggs and swim-up fry for the 1st and 2nd production cycles is presented in Table 7. The swim-up fry collected in the 1st production was added to the number of the eggs collected; it was assumed to have a 100% hatching rate.

Breeders from LSRHSR♂♀ produced the highest mean number of eggs for both cycles having 3260.33 and 1883.67 eggs, respectively. It was followed by those in LSR♂♀ with 2484.33 eggs in the 1st cycle and 1582.33 eggs in the 2nd cycle. The breeders in LSR♂HSR♀ obtained a higher mean egg collection of 1557.33 eggs in the 2nd cycle than in its 1st production cycle which was just 867.33 eggs. This same trend was also shown in HSR♂LSR♀. On the other hand, breeders in HSR♂♀ generated 2142.00 eggs in the 1st production, however, this figure was only a representation of just one replicate. No eggs were collected in the other two replicates. And for the 2nd production, no eggs had been collected in all the replicates, thus, obtaining a zero (0) value. Only 1 replicate in HSR♂♀ was able to produce eggs during the 1st cycle and none in the 2nd. Three breeders had died in the HSR♂♀ group a day before the scheduled collection of the 2nd cycle; two from replicate one (one male and one female) and one female from replicate three.

On the number of eggs collected no significant differences were observed in LSRHSR♂♀, LSR♂♀, and HSR♂♀. The mean number of eggs collected in LSR♂HSR♀ and HSR♂LSR♀ were also comparable to each other, but were significantly lower than those of the first three treatments. For the 2nd production cycle, since no eggs were collected in HSR♂♀, analysis was done based on the log (x+1) transformation. Comparison among treatment means showed that those in LSRHSR♂♀, LSR♂♀, LSR♂HSR♀ and HSR♂LSR♀, were all comparable among each other but significantly higher than that in HSR♂♀.

Total seed produced in LSRHSR♂♀ (5144.00) was comparable to that in LSR♂♀ (4016.66), but was significantly higher than those of the other treatments (Table 8). Total seed production in the LSR♂♀ group was significantly higher than that of HSR♂♀, but comparable to those of the LSR♂HSR♀ and HSR♂LSR♀. HSR♂♀, LSR♂HSR♀, and HSR♂LSR♀ had comparable total seed production over the two cycles.

Hatching Rate: The eggs in LSR♂♀ reflected the highest hatching rates of 92.44% and 84.13% for 1st and 2nd production, respectively (Table 9). It was followed by LSR♂HSR♀ with 87.68% for the 1st production and 83.60% for the 2nd production. The LSRHSR♂♀ although having the highest number of eggs collected dropped to 3rd, as it only showed 78.26% and 83.56% hatching rates. This finding suggests that most of the eggs collected in LSRHSR♂♀ died before they hatched. Significance on the comparison among treatment means was seen only in HSR♂♀ in 1st and 2nd productions. While LSRHSR♂♀, LSR♂♀, LSR♂HSR♀ and HSR♂LSR♀ were comparable with no significant differences.

Survival Rate: The highest mean survival rate was attained by post-fry in LSR♂♀ with 81.83% in the 1st cycle and 78.14% in the 2nd cycle (Table 10). While, the lowest rate was obtained by post-fry in HSR♂♀ with 47.53% in the 1st cycle. The post-fry in LSRHSR♂♀, LSR♂HSR♀ and HSR♂LSR♀ on the other hand, obtained average mean survival rates of 68.64%, 66.46%, and 58.44%, respectively, in both cycles. The HSR♂♀ was significantly lower than the other treatments. While LSR♂♀, LSR♂HSR, LSRHSR♂♀ and HSR♂LSR♀ had comparable survival rates and revealed no significant differences. In the 2nd cycle, the survival rate of post fry in LSR♂♀, LSRHSR♂♀ and LSR♂HSR♀ were comparable to each other and significantly higher than that of HSR♂♀. However, that of HSR♂LSR♀ was comparable to that of LSR♂HSR♀, but was significantly higher than that of HSR♂♀. Campbell et al. (1994) found that a relatively prolonged and severe stress in rainbow trout negatively affects progeny survival. The reduced progeny viability might be attributed to limited energetic reserves allocated to the eggs as well as

mechanical damage caused by the stressor (Schreck 2000). In this study, treatments with LSR males as in LSRHSR♂♀, LSR♂♀ and LSR♂HSR♀ showed better survival percentages than in the treatments with HSR males (HSR♂♀ and HSR♂LSR♀). Highest survival rate was attained in treatment with both LSR male and female breeders. The quality of breeders may have contributed to the quality of gametes produced and resulted in better quality fry which led to higher rates of survival. The lowest survival rate of post-fry was attained in the treatment with both HSR male and female breeders.

Fry Length and Weight: After three weeks of rearing period, the swim-up fry were collected from the nursing hapas. The final length and weight of the post-fry in each treatment after three weeks are presented in Tables 11 and 12. Post-fry in LSR♂♀ group had the highest measured mean length of 20.91 and 20.34 mm for both production cycles, respectively. This was followed by those in HSR♂LSR♀ which had 19.38 mm in the 1st cycle and 19.24 mm in the 2nd. Post-fry in LSRHSR♂♀ and LSR♂HSR♀ on the other hand, obtained 18.98 mm and 18.32 mm in the 1st production, while 18.37 mm and 18.29 mm in the 2nd production, respectively. The lowest value was obtained in HSR♂♀ which was 13.88 mm in the 1st production. The LSR♂♀ had significantly higher mean length than other treatments. The LSRHSR♂♀, LSR♂HSR♀ and HSR♂LSR♀ had comparable post fry lengths but were significantly higher than that in HSR♂♀. As for the 2nd cycle, mean length in the LSR♂♀ group was significantly higher than those of the other social groups. The length of HSR♂LSR♀ fry was also significantly higher than those of the remaining three treatments. Whereas, the mean length in LSRHSR♂♀ and LSR♂HSR♀ were comparable to each other but were significantly higher than that in HSR♂♀. Similar differences in mean weight of fry was also observed among the groups.

Overall, the highest length and weight was seen in the post-fry produced by both LSR breeders (LSR♂♀). The quality of the progeny produced is likely a reflection of broodstock quality. The LSR breeders during the isolation period showed high feeding response scores, and take a shorter period adjusting to stress as exhibited by immediate aggressiveness towards the feed provided. Along with this result, it was observed that good numbers can also be acquired as long as both the breeders were not under the HSR group. In this study, HSR♂LSR♀ with low stress response females and high stress response males produced bigger post-fry after three weeks than in LSRHSR♂♀ with equal combination of low and high stress response male and female breeders and LSR♂HSR♀ with low stress response males and high stress response females. This trend was probably influenced by the quality of female breeders having the low stress response as determined through feeding response observations.

Study 3 – Effect of Broodstock Social Condition on the Growout Performance of Nile Tilapia Fingerlings

The effect of the social condition on the grow-out performance of Nile tilapia fingerlings were evaluated by monitoring growth and performance of the fish stocks in earthen ponds produced from broodfish that show a low stress response (high feeding response scores during isolation) and high stress response (low feeding response scores during isolation). Figure 6 shows the growth pattern of both treatments in terms of the average body weight of the fish stock during the culture period.

The figure shows a comparable growth between treatments during the first month of the culture period but treatment means started to differ on the second month until the end of the experiment. Fish stock from Treatment I which was from the broodstock with low stress response obtained a higher final mean weight of 111.27 g as compared to those produced from the high response group with a final mean weight of 90.31 g. However, analysis of variance showed no significant d ifference among treatments at 5% level of significance (Table 13).

During the growout period, fish produced from the broodstock with low stress response generally obtained better results as compared to the fish produced from high stress response. In terms of feed

conversion ratio (FCR), Treatment I had a lower FCR of 1.4 as compared to Treatment II with 1.7 at the end of the culture period. The low stress response group also had the higher extrapolated gross yield (1596.7 kg/ha versus 1221.7 kg/ha. On the other hand, survival of both treatments was almost identical. Despite the trends toward improved performance in fish derived from low stress response broodfish, the difference was not statistically significant, at least, within the 3-month culture period.

Monitoring of water quality parameters was carried out throughout the duration of the study and results are presented in Table 14. The data gathered for dissolved oxygen in both treatments were in the ideal range for tilapia culture, although minimum reading of 1.8 was recorded, still, tilapia are known to tolerate low level of dissolved oxygen level but can affect growth if exposure occurs over a long period (Boyd 1990). The minimum and maximum readings for all other water parameters, including temperature, pH, ammonia-nitrogen, alkalinity, and phosphorus were desirable and similar between treatment groups. This is further underscored by the limited mortality seen in the experiment (> 70% survival).

Study 4: Effect of Stocking Density on the Growth Responsiveness, and IGF-1 Expression on Nile Tilapia

Water Quality: The temperature readings obtained in this experiment were within the optimal range of 20 to 35 °C. The morning dissolved oxygen concentrations ranged from 2.36 to 4.14 mg/l while the afternoon readings of dissolved oxygen ranged from 2.04 to 7.89 mg/l. The Carlson's technique of log transformation and calculation of the average Secchi disk readings gave a TSI of 63.95, which indicates the pond used in this study had sufficient nutrients to support fairly high natural productivity.

Growth Parameters and Survival: Survival rates ranging from 61 to 70% did not differ among sex-reversed tilapia reared at the different densities. Most of the observed mortalities occurred over the first 10 days following stocking.

At the initiation of the study average body weight and variation in weight was highest for the low density mixed sex group (control) relative to all other groups that consisted of sex reversed fish at similar or higher stocking densities. At harvest, the average final weight declined with increased stocking density. The low density (250 fish/m^3), mixed-sex (T1, 9.0 g) and sex-reversed (T2, 6.01g) had similar final body weights with significantly lower values for sex-reversed fish stocked at 500 (T3, 4.93 g) and 1000 (T4, 4.66 g) fish/m^3 ($P < 0.05$; Table 15). The SGR of fish at different sampling periods in response to stocking density-related stress are shown in Table 16. The low density, sex-reversed fish (T2) had highest SGR over the course of the experiment. Graded declines in SGR was seen as stocking density increased. This difference was statistically significant during the second sampling interval (day 10-24). The overall SGR was 8 - 10 times greater in low density sex-reversed fish relative to the medium and high density group. Interestingly, the mixed-sex low density control (T1) had a lower SGR than the low density sex-reversed male fish, suggesting energy may have been diverted to both gonadal development, particularly for females, as well as to growth in the mixed-sex population. The SGR observed in this study with lower density conforms with that shown previously (SGR of 1.7) in monosex fish reared in hapas (Little et al. 2003). The findings of Chakraborty and Banerjee (2010) on SGR of fish in ponds was higher at 5.01%, in cages at 4.68%, and in cisterns at 4.8% with additional dietary protein sources.

Data generated in this study demonstrated that fish stocked at the lowest density had better growth performance in terms of average weight and SGR over a 30-day nursery period. This was followed by sex-reversed fish stocked at the medium density with lowest performance occurring in fish stocked at the highest density. The results suggest that at higher densities the carrying capacity of fish may be limited due to space, which in turn, elicits a stress response accompanied by lower growth rate.

Hepatic IGF-1 Gene Expression: Baseline hepatic IGF-1 concentrations were recorded from the experimental fish reared in normal conditions. The IGF-1 mRNA concentrations of Nile tilapia were as follows: female in hapa (30.52 ng/µl), male in hapa (M-H, 31.5 ng/µl), sex-reversed in hapa (SR-H, 30.67 ng/µl) and sex-reversed in pond (SR-P, 29.31ng/µl). These IGF-1 mRNA concentrations served as pre-stress reference values for the succeeding IGF-1 evaluations in fish from the density study.

The levels of hepatic IGF-1 mRNA in Nile tilapia following 7 days (March 16 sampling) of rearing at different stocking densities are shown in Table 17. Hepatic IGF-1 mRNA levels was highest in low density, sex-reversed fish (T2, 31.59 ± 6.94 ng/µl) followed by the control, low density mixed-sex (T1, 27.03 ± 5.24 ng/µl). These levels were similar to those pre-stress baseline values measured in fish in hapas and ponds ($P > 0.05$). Significantly lower IGF-1 mRNA concentrations were found in medium and high stocking densities with 14.44 ng/ µl ± 10.90 and 17.79 ng/µl ± 7.64, respectively ($P < 0.05$). Due to technical difficulties and/or RNA degradation we were unable to detect any IGF-1 mRNA taken from liver on the 15 day midpoint and 30-day endpoint sampling. A post-hoc analysis in low density, sex-reversed fish (T2) on pre-stress and post-stress comparison showed that there was no significant increase in the level of IGF-1 mRNA ($P>0.05$).

Insulin-like growth factor -1 (IGF-1) is the primary mediator of the growth promoting actions of growth hormone (GH) and its levels correlate well with growth in tilapia and other fishes and vertebrates (Picha et al. 2008a). It is an indirect measure of the average amount of growth hormone (GH) being produced by the body, thus, IGF-1 mirrors GH excesses and deficiencies, making it a useful indicator of average GH levels (American Association for Clinical Chemistry 2011). The lower hepatic IGF-1 gene expression in fish reared at the medium and high densities along with their reduced growth rates clearly indicates that density-dependent stress is likely suppressing growth through inhibition of IGF-1 and perhaps GH cell activity. Brockmark et al. (2007) showed that salmonid smolts maintained at low density had higher levels of IGF-1 than those reared at high density, which is consistent with the results shown here. They further pointed out that fish kept at low density were more silvery in color and had a lower mortality rate than fish reared at high density.

Hepatosomatic and Cholecystic Indices: The liver of fish from the control, low density mixed-sex (T1) had the highest weight of 0.20 g, followed by low density, sex-reversed (T2, 0.11 g; $P < 0.05$; Table 18). The liver weight of fish from the medium density, sex-reversed (T3) and high density, sex-reversed (T4) was significantly lower at 0.08 and 0.07 g, respectively compared with low density fish. The length of liver in the control, low density, mixed-sex (T1) was longer at 3.24 mm compared to sex-reversed fish reared at the different densities ($P < 0.05$), a likely reflection of the larger body size of this fish both at the beginning and end of the experiment. There were no differences in liver length among sex-reversed fish held at the different densities. The HSI or ratio of liver weight to body weight, is a general indicator of energy reserves, namely glycogen and fat. Fish tend to have a lower HSI in a poor environment where limited nutrients might be available or where excessive energy is utilized. In gilthead seabream high stocking density reduces hepatosomatic index (from 2.26 down to 2.04)(Montero et al. 1999). The HSI values ranged from 0.961 to 1.10 in this study and there were no significant differences in the across density classes ($P > 0.05$), suggesting energy (or food) was not a limiting factor or that metabolic rate was not sufficiently elevated in fish held at higher densities, despite their lower growth rate.

The size and fullness of the gall bladder is indicative of feeding status in fish. A large, distended bladder indicates a fish that has not eaten for some time while an empty, flabby bladder suggests a recent meal (Bowen 2001). An elevated cholecystic index (gall bladder:liver weight ratio) may be suggestive of bile retention, a decrease flow of bile and therefore susceptibility to cholelithiasis. Although gall bladder weight was slightly elevated in the mixed-sex, low density fish, no differences were observed in weight, length or cholecystic index among the sex-reversed fish held at different densities (Table 18). The

highest cholecystic index of 41.3% was recorded in high density, sex-reversed (T4) fish, but this was not significantly different from the other groups.

Blood Glucose: Blood glucose levels generally rise with stress in most vertebrates to provide the necessary energy for metabolism, muscle activity and other functions needed for short term and long term adaptation to stress or the fight-or-flight response. In tilapia social stress leads to elevations in glucose relative to pre-stress levels (Porchas et al. 2009). Likewise, in sturgeon glucose levels rise following a stressor similar to that observed for bass with temperature and confinement stress (Solati and Falahatkar 2007; Porchas et al. 2009). However some studies in fish reported a weak elevation of glucose (Davis and McEntire 2006), while others found no change (Jentoft et al. 2005) and even a decrease in glucose levels (Wood et al. 2005). In the present study, sex-reversed tilapia stocked at the medium density had signifcantly higher levels of blood glucose (94.50 mg/dl) relative to the low density (62.75 mg/dl) and high density (49.75 mg/dl), sex-reversed groups ($P < 0.05$; Table 19). The use glucose as a putative marker of density-dependent stress in tilapia may be inconclusive because tilapias tend to tolerate low glucose levels and maintain their blood glucose within a relatively narrow range. This may be attributed to tilapia being omnivorous tropical species as compared to carnivorous cold water species. Alternatively, we may have missed potential changes in glucose that may have occurred in response to the initial effects of stocking density.

Hematological Profiles: Total red blood cell (RBC) and white blood cell (WBC) profiles in response to different rearing densities are shown in Table 20. Fish in the control, low density, mixed-sex group had the highest RBC count of 3.7×10^9 cells/ml of blood ($P < 0.05$). The RBC count in sex reversed fish declined in parallel with elevations in density, such that the low density group had an RBC count of 2.85×10^9 cells, the medium density 2.45×10^9 cells, and the high density 2.0×10^9 cells per ml of blood ($P < 0.01$). A virtually identical pattern was observed with WBC counts. Clearly, RBC and WBC counts decline with increasing density. A decrease of RBC count most likely suggests that higher density fish lack sufficient oxygen or our anemic despite adequate amounts in rearing water. This may contribute to lower growth rate associated with the stressor of higher densities. The variation in total WBC clearly indicates it is a good measure of density dependent stress responses. The lower WBC count with higher density may reflect release of epinephrine during stress which causes contraction of spleen and could hasten WBC destruction. Destruction of WBC may weaken of the immune system (Witeska 2005). Thus the high density, sex-reversed fish group which apparently were subjected to the most stressful condition in the course of the experiment, and had the lowest WBC count, may have a weakened immune system, which could enhance vulnerability to infection. It appears since the survival rates were similar among groups that this downstream effect was not fully apparent within the 30-day culture period. Had the culture period been extended perhaps mortality rates would have risen in fish at the higher densities

CONCLUSION

Through a series of studies we assessed if physiological and/or behavioral responses to stress can be used in the selection of broodstock with reproductive advantage in Nile tilapia, examined the effect of broodstock social condition on seed production and fingerling growout performance of tilapia, and evaluated the effects of stocking density on fingerling growth in nursery hapas, gene expression of IGF-1, and stress responsiveness of tilapia.

In the first study, we investigated whether the outcomes the outcomes of competition for social dominance among Nile tilapia individuals can be predicted by evaluating the duration of appetite inhibition or the feeding response score (FRS) after transfer to isolation. In addition, it also investigates if eye color pattern (ECP) is related to the duration of behavioral stress response such as appetite inhibition. Clear establishment of dominance hierarchy was observed in 24 of the 25 pairs. From the 24 dominants, 17 (70.83%) of them have shorter DAI during isolation compared to that of their conspecifics. This

indicates that tilapia with shorter DAI during the isolation had a greater possibility to win the fight for social dominance and therefore, dominance can be predicted using the DAI of the fish during isolation. Reduced growth rate of both dominant and subordinate fish, a well-described physiological end result of social stress, were observed one day after the social interaction. The greater weight losses in subordinate fish compared to dominant fish during and after the establishment of social hierarchy were mainly attributed to behavioral differences such as appetite rather than to differences in physical activities. Similarly, we found that animals with a higher FRS could be used as a predictor of social dominance. Again, the dominant fish had higher specific growth rates, which were accompanied by elevated expression of IGF-1, a central hormone mediating growth in tilapia and other vertebrates. Based on this research, feeding responses of broodstock in isolation are good predictors of social status, such that dominant individuals can be chosen in establishing breeding pairs.

In study 2, we assessed the effect of broodfish behavioral stress response on seed production of tilapia through evaluation of their feeding response score during isolation.

Results of the study demonstrate that behavioral stress response of Nile tilapia through evaluation of their feeding response can influence the number and quality of the seeds they produce. The most number of eggs, largest egg size, highest sperm motility and sperm density, and most number of fingerlings produced were collected in treatments that had both low stress response male and female breeders in the group. Higher rates of hatching and survival were also reflected in treatments that had either a low stress response male or female in the group. These results indicate that stress responsiveness of broodstock is a good predictor of fecundity and can be used to select fish with higher seed production. Broodstock individuals with low stress responsiveness, selected based on their increased feeding responses during an initial isolation period, can improve overall seed production in hatchery operations.

A third study the effect of social condition of broodfish on grow-out performance of Nile tilapia fingerlings was evaluated. We found that a 3-month growout of sex-reversed fingerlings derived from low stress response breeders, had a higher average body weight, a better feed conversion ratio, and an overall higher yield per hectare than fingerlings derived from high stress response breeders. Although the differences between the two groups were not statistically significant after 3 months of growout the data suggests that fingerlings derived from low stress response broodstock pose an advantage over those derived from high stress response broodstock in overall production performance. This would likely be further amplified were fish growout extended to 4 or 5 months.
An additional study investigated the effect of fingerling stocking density on the growth, gene expression of hepatic insulin-like growth factor-I (IGF-I), and stress responsiveness of tilapia reared in nursery hapas. The overall effect of density as a stressor showed that low density, sex-reversed group responded well in terms of growth, specific growth rate, survival, hematological profile (elevated red blood and white blood cell counts) and IGF-1 mRNA gene expressions compared to fish reared and confined at higher densities. These data suggest that densities of 250 fish/m^3 are best for growth of fingerlings in nursery hapas, and demonstrate that density-dependent stressors impair growth through inhibition of IGF-1 production. It is clear that IGF-1 mRNA is a strong growth rate indicator in the field and may also serve as an indirect measure of stress in tilapia. This along with certain hematological parameters may allow for assessment of environmental variables that limit stress and best promote growth in tilapia aquaculture.

LITERATURE CITED

Barcellos, L.J.G., Nicolaiewsky, S., de Souza, S.M.G. and Lulhier, F. 1999. Plasmatic levels of cortisol in the response to acute stress in Nile tilapia, *Oreochromis niloticus* (L.), previously exposed to chronic stress. Aquac. Res. 30: 437-444.

Barton, B.A. 2002. Stress in fishes: a diversity of responses with particular reference to changes in circulating corticosteroid. Integ. Comp. Biol. 42: 517-525.
Bearden, H.J. and Fauquay, J.W. 1980. Applied Animal Reproduction. New Jersey, USA: Prentice Hall.
Bernier, N.J. 2006. The corticotropin-releasing factor system as a mediator of the appetite-suppressing effects of stress in fish. General and Comparative Endocrinology, 146: 45-55
Bero, R.M.L. 2008. Eye color as a predictor of social dominance in Nile tilapia (*Oreochromis niloticus* L.). Undergraduate Thesis. Central Luzon State University. 53 pp.
BFAR. 2006. The Bureau of Fisheries and Aquatic Resources: Overview. Paper presented at the WorldFish Center and Philippine Partners meeting. Quezon City, Philippines.28 February 2006.
Binuramesh, C., Prabakaran, M., Steinhagen, D. and Michael, R.D. 2005. Effect of chronic confinement stress on the immune responses in different sex ratio groups of *Oreochromis mossambicus* (Peters). Gen. Comp. Endocrinol.. 250, 47-59.
Björnsson, B.T. 1994. Effects of stocking density on growth rate of halibut (*Hippoglossus hippoglossus* L.) reared in large circular tanks for three years. Aquaculture 123: 259-270.
Blanchard, D.C., Sakai, R.R., McEwen, B., Weiss, S.M. and Blanchard, R.J. 1993. Subordination stress: behavioural, brain and neuroendocrine correlates. Behavioral Brain Research, 58: 113-121.
Blanchard, D.C., Spencer, R.L., Weiss, S.M., Blanchard, R.J., McEwen B. and Sakai, R.R. 1995. Visible burrow system as a model of chronic social stress: behavioral and neuroendocrine correlates. Psychoneuroendocrinology, 20: 117- 134
Bolasina, S., Tagawa, M., Yamashita, Y. and Tanaka, M. 2006. Effect of stocking density on growth, digestive enzyme activity and cortisol level in larvae and juveniles of Japanese flounder, *Paralichthys olivaeus*. Aquaculture 259: 432-443.
Bonga S.E.W. 1997. The stress response in fish. Physiol. Rev. 77 (3):591-625
Bowen, R. 2001. Secretion of bile and the role of bile acids in digestion. Retrieved on June 27, 2011 from http://www.vivo.colostate.edu/hbooks/pathphys/digestion/liver/bile.html.
Boyd, C.E. 1990. Water Quality in Ponds for Aquaculture. Alabama Agriculture Experiment Station, Auburn University, Alabama, USA.
Brockmark, S.L., Bohlin, N.T., Bjornsson, B.T. and Johnson, J.L. 2007. Effect of rearing density and structural complexity on the pre and post release performance of Atlantic salmon. Transaction of the American Fisheries Society 136:1453-1462.
Burmeister, S.S., Kailasanath, V. and Fernald, R.D. 2007. Social dominance regulates androgen and estrogen receptor gene expression. Hormones and Behavior 51: 164 – 170.
Campbell, P.M., Pottinger, T.G. and Sumpter, J.P. 1994. Preliminary evidence that chronic confinement stress reduces the quality of gametes produced by brown and rainbow trout. Aquaculture 120: 151–169.
Chakraborty, S.B. and Banerjee S. 2010. Effect of stocking density on monosex Nile tilapia growth during pond culture in India. World Academy of Science, Engineering and Technology 68:1511-1515.
Chandroo, K.P., Duncan, I.J.H. and Moccia, R.D. 2004. Can fish suffer?: perspectives or sentience, pain, fear and stress. Appl. Anim. Behav. Sci. 86: 225-250
Contreras-Sanchez, W.M., Schreck, C.B., Fitzpatrick, M.S. and Pereira, C.B. 1998. Effects of stress on the reproductive performance of rainbow trout (*Oncorhynchus mykiss*). Biology and Reproduction 58: 439 – 447.
DiBattista, J.D., Levesque, H.M., Moon, T.W. and Gilmour, K.M. 2006. Growth depression in socially subordinate rainbow trout *Oncorhynchus mykiss*: more than a fasting effect. Physiol. Biochem. Zool. 79: 675-687.
Danting, M.J.C. 1992. Optimization of UV radiation treatment and cryopreservation of *O. niloticus* spermatozoa for induced gynogenesis. Unpublished Masteral thesis. Institute of Aquaculture, University of Stirling, Stirling Scotland. p. 74.
Davis, K.B. and McEntire, M.E. 2006. Effect of photoperiod on feeding, intraperitoneal fat, and IGF-I in sunshine bass. Annual Meeting World Aquaculture Society 37:431-436.

Dean, D.B., Whitlow, Z. and Borski R.J. 2003. Glucocorticoid receptor upregulation during seawater adaptation in a euryhaline teleost, the tilapia (*Oreochromis mossambicus*). Gen. Comp. Endocrinol. 132:112-118.

Degger, B., Upton, Z., Soole, K., Collet, C. and Richarson, N. 2000. Comparison of recombinant barramundi and human insulin-like growth factor (IGF)-I in juvenile Barramundi (*Lates calcarifer*): *In vivo* metabolic effects, association with circulating IGF-binding proteins, and tissue localization. Gen. Comp. Endocrinol. 117: 395-403.

Doyon, C., Gilmour, K.M., Trudeau, V.L. and Moon, T.W. 2003. Corticotropin-releasing factor and neuropeptide Y mRNA levels are elevated in the preoptic area of socially subordinate rainbow trout. General and Comparative Endocrinology, 133: 260-271.

Earley, R.L., Blumer, L.S. and Grober, M.S. 2004. The gall of subordination: changes in gall bladder function associated with social stress. Proc. R. Soc. Lond. 271: 7-13.

El-Azab, A.I., Adel-Ghafar, A.E., Nasr, M.T. and Issa, E.S. 1996. Effect of exogenous PGF2alpha and/or GnRH on libido and semen production of aged Friesian bulls. Journal of Veterninary Medicine 44 : 529-533.

Fox, H.E., White, S.A., Kao, M.H.F., and Fernald, R.D. 1997. Stress and dominance in a social fish. Journal of Neuroscience, 17: 6463-6469.

Gabor, G., Sasser, R.G., Kastelic, J.P., Coulter, G.H. Everson, D.O., Falkay, G., Meezes, M., Bozo, S., Cook, R.B., Volgyi, J.C., Barany, I. and Szaasz, F. 1998. Endocrine and thermal responses to GnRH treatment and prediction of sperm output and viability in Holstein-Friesian breeding bulls. Theriogenology 50: 177 – 183.

Gómez-Laplaza, L.M. and Morgan, E. 2003. Theinfluence of social rank in angelfish, *Pterophyllum scalare*, on locomotor and feeding activities in a novel environment. Laboratory Animals, 37: 108-120.

Goncalves-de-Freitas, E. 1999. Investimento reprodutivo e crescimento em machos de tilapia-do-Nilo. Tese de doutorado. Instituto de Biociencias, Universidade Estadual Paulista, Botucatu, SP.

Höglund, E., Kolm, N. and Winberg, S. 2001. Stress-induced changes in brain serotonergic activity, plasma cortisol and aggressive behavior in Arctic charr (*Salvelinus alpinus*) is counteracted by L-DOPA. Physiological Behavior, 74: 381-389.

Holfman, H.A. and Fernald, R.D. 2000. Social status controls somatostatin neuron size and growth. J Neurosci. 20: 4740-4744.

Jentoft, S., Topp, N., Seeliger, M., Malison, J.A., Barry, T.B., Held, J.A., Roberts S. and Goetz, F. 2005. Lack of growth enhancement by exogenous growth hormone treatment in yellow perch (*Perca flavescens*) in four separate experiments. Aquaculture, 250: 471–479.

Maeda, K. and Tsukamura, H. 2006. The impact of stress on reproduction: are glucocoticoids inhibitory or protective to gonadotropin secretion. Endocrinology 147: 1085-1086.

Montero, D., Izquierdo, M.S., Tort, L., Robaina L. and Vergara, J.M. 1999. High stocking density produces crowding stress altering some physiological and biochemical parameters in gilthead seabream, *Sparus aurata*, juveniles. Fish Physiology and Biochemistry 20:50-60.

Mostl, E. and Palme, R. 2002. Hormones as indicators of stress. Domestic Animal Endocrinology, 23:67-74.

Koolhaas, J.M., Korte, S.M., De Boer, S.F., Van Der Vegt, B.J., Van Reenen, C.G., Hopster, H., De Jong, I.C., Ruis, M.A.W. and Blokhuis, H.J. 1999. Coping styles in animals: current status in behavior and stress-physiology. Neurosci. Biobehav. Rev. 23: 925-935.

Korzan, W.J., Ø. Øverli, and Summers, C.H. 2006. Future social rank: Forecasting status in the green anole (*Anolis carolinensis*). Acta Ethology, 9: 48-57.

Korzan, W.J., Robison, R.R., Zhao, S. and Fernand, R.D. 2008. Color change as potential behavioral strategy. Horm. Behav. 54: 463-470.

Kurokura, H. and Oo, K.M. 2008. Evaluation of fertilizing capacity of cryopreserved rainbow trout sperm. Fisheries Science 74: 621 – 626.

Little, D.C. and Bhujel, R.C. 2003. Advanced nursing of mixed-sex and mono-sex tilapia (*Oreochromis niloticus*) fry, and its impact on subsequent growth in fertilized ponds. Aquaculture 221:265–2.

Øverli, Ø., Winberg, S., Damsgard, B. and Jobling, M. 1998. Food intake and spontaneous swimming activity in Arctic charr (*Salvelinus alpinus*): role of brain serotonergic activity and social interactions. Canadian Journal of Zoology, 76: 1366-1370.

Øverli, Ø., Harris, C.A. and Winberg, S. 1999. Short-term effects of fights for social dominance and the establishment of dominant-subordinate relationship on brain monomines and cortisol in rainbow trout. Brain Behavior and Evolution, 54: 263-275.

Øverli, Ø., Korzan, W.L., Höglund, E., Winberg, S., Bollig, H., Watt, M., Forster, G.L., Barton, B.A., Øverli, E., Renner, K.J. and Summers, C.H. 2004. Stress coping style predicts aggression and social dominance in rainbow trout. Hormones and Behavior, 45: 235- 243.

Øverli, Ø., Sorensen, C., Pulman, K.G.T., Pottinger, T.G., Korzan, W., Summers C.H. and Nilsson, G.E. 2007. Evolutionary background for stress-coping styles: relationships between physiological, behavioural, and cognitive traits in non-mammalian vertebrates.neuroscience and Biobehavioral Reviews, 31: 396-412.

Payne, A.I. and Collimson, R.I. 1983. A comparison of the biological characteristics of *Sarotherodon niloticus* (L.) with those of *S. aureus* (Steindachner) and other tilapia of the delta and lower Nile. Aquaculture 30: 335 – 351.

Petrauskiené, L. 1996. Effects of social stress in rainbow trout at different stocking densities. (www-heb:pac.dfo-mpo.gc.ca/congress/1996/Applied/Petrauskiene1.pdf.)

Picha, M.E., Silverstein, J.T. and Borski, R.J. 2006. Discordant regulation of hepatic IGF-I mRNA and circulating IGF-I during compensatory growth in a teleost, the hybrid striped bass (Morone chrysops X M. saxatilis). Gen. Comp. Endocrinol. 147:196-205.

Picha, M.E., Turano, M.J. Beckman, B.R. and Borski, R.J. 2008a. Hormonal biomarkers of growth and applications to aquaculture: A mini-review of growth hormone, insulin-like growth factor-I and IGF binding proteins as growth indicators in fish. NAJA 70: 196–211.

Picha, M.E., Turano, M.J., Tipsmark, C.K. and Borski, R.J. 2008b. Regulation of endocrine and paracrine sources of insulin-like growth factors and growth hormone receptor during compensatory growth in a teleost fish. J. Endocrinol. 199:81-94.

Porchas, M.M., Cordova, L.R.M. and Enriquez, R.R. 2009. Cortisol and glucose: reliable indicators of fish stress? Pan-American Journal of Aquatic Sciences, 4(2): 158-178.

Pottinger, T.G. and Carrick, T.R. 2001. Stress responsiveness affects dominant-subordinate relationships in rainbow trout. Hormones and Behavior, 40: 419-427.

Parikh, V.N., Clement, T.S. and Fernald, R.D. 2006. Androgen level and male social status in African cichlid, *Astatotilapia burtoni.* Behavioral Brain Research 166: 291 – 295.

Schreck, C.B. 2000. Accumulation and long-term effects of stress. In: Moberg, G.P., Mench, J.A. (Eds.), The Biology of Animal Stress: Assessment and Implications for Welfare. CAB International, Wallingford.

Siegfried, B., Frischknecht, H.R. and Waser, P.G. 1984. Defeat, learned submissiveness, and analgesia in mice: effects of genotype. Behaviour of Neural Biology, 421: 91-97.

Sloman, K.A. Gilmour, K.M., Taylor, A.C. and Metcalfe, N.B. 2000. Physiological effects of dominance hierarchies within groups of brown trout, Salmo trutta, held under simulated natural conditions. Fish Physiol. Biochem., 22:11-20

Sodeberg, R.W. and Meade, J.W. 1987. Effects of rearing density on growth survival and fin condition of Atlantic salmon. Prog. Fish-Cult. 49: 280-283.

Solati, N.H and Falahatkar, B. 2007. Stress responses in sub-yearling great sturgeon to the air exposure, Caspian Journal of Environmental Sciences 5:99-103.

Subasinghe, R.P. and Sommerville, C. 1992. Effects of temperature on the hatchability, development and growth of eggs and yolksac fry of *Oreochromis mossambicus* (Peters) under artificial incubation. Aquaculture and Fisheries Management 23: 31 – 39.

Vahdatpour, T., Nazer Adl, K., Nezhad, Y.E., Sis, N.M., Riyazi S.R. and Vahdatpour S. 2009. Effects corticosterone intake as stress-alternative hormone on broiler chickens: Performance and blood parameters. Asian Journal of Animal & Veterinary Advances, 4(1):16-21. Academic Journals Inc.

Vera Cruz, E.M., Bero, R.M.L., Bolivar, R.B. and Borski, R.J., 2009. Eye color predicts social dominance in Nile Tilapia *Oreochromis niloticus*. Paper presented at the WAS AQUA 2009, Seattle, Washington, USA. February 14-18, 2009.

Vera Cruz, E.M. and Brown, C.L. 2007. The influence of social status on the rate of growth, eye color pattern and insulin-like growth factor-I gene expression in Nile tilapia, *Oreochromis niloticus.* Horm. and Behav. 51: 611-619.

Vera Cruz, E.M., Brown, C.L., Luckenbach, J.A., Picha, M.E., Bolivar, R.B. and Borski, R.J. 2006. Insulin-like growth factor-I cDNA cloning, gene expression and potential use as a growth rate indicator in Nile tilapia, *Oreochromis niloticus*. Aquaculture 251: 585-595.

Volpato, G.L., Luchiari, A.C., Duarte, C.R.A., Barreto, R.E. and Ramanzini, G.C. 2003. Eye color as an indicator of social rank in the fish Nile tilapia. Braz. J. Med. Biol. Res. 36: 1659-1663.

Winberg, S., Nilsson, G.E. and Olsén, K.H. 1992. Changes in brain serotonergic activity during hierarchic behavior in artic charr (*Salvelinus alpinus* L.) are socially induced. Journal of Comparative Physiology, 170, 93-99.

Winberg, S., Carter, C.G., McCarthy, I.D., He, Z.-Y., Nilsson G.E. and Houlihan, D.F. 1993. Feeding rank and brain serotonergic activity in rainbow trout *Oncorhynchus mykiss*. Journal of Experimental Biology, 179: 197-211.

Wood, A.W., Duan, C. and Bern, H.A. 2005. Insulin-like growth factor signaling in fish. International Review of Cytology, 243:215-85.

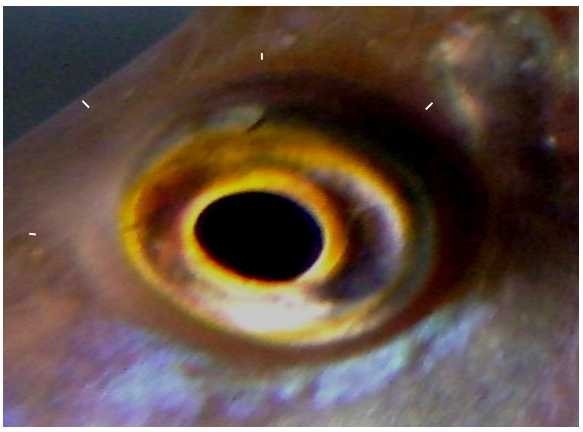

Figure 1. Eye color pattern of Nile tilapia.

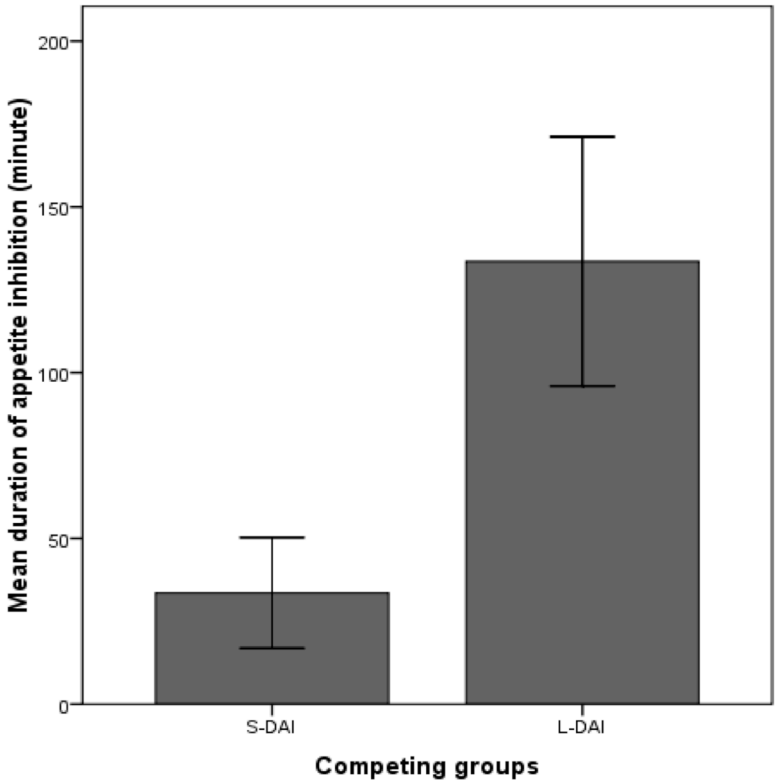

Figure 2. Average duration of appetite inhibition (minute) of the two competing groups. S-DAI, short DAI group; L-DAI, long DAI group. Average DAI were significantly different at $P < 0.01$. Mean ± SEM

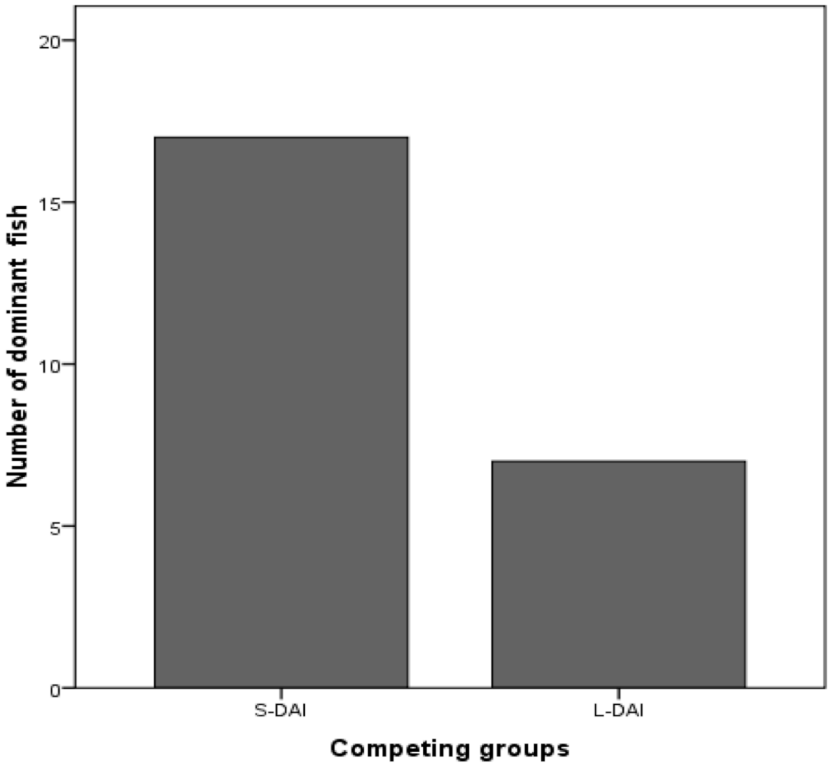

Figure 3. Number of dominant fish in the two competing groups. S-DAI: short DAI group; L-DAI: long DAI group. Frequency difference was significantly different at $P < 0.05$.

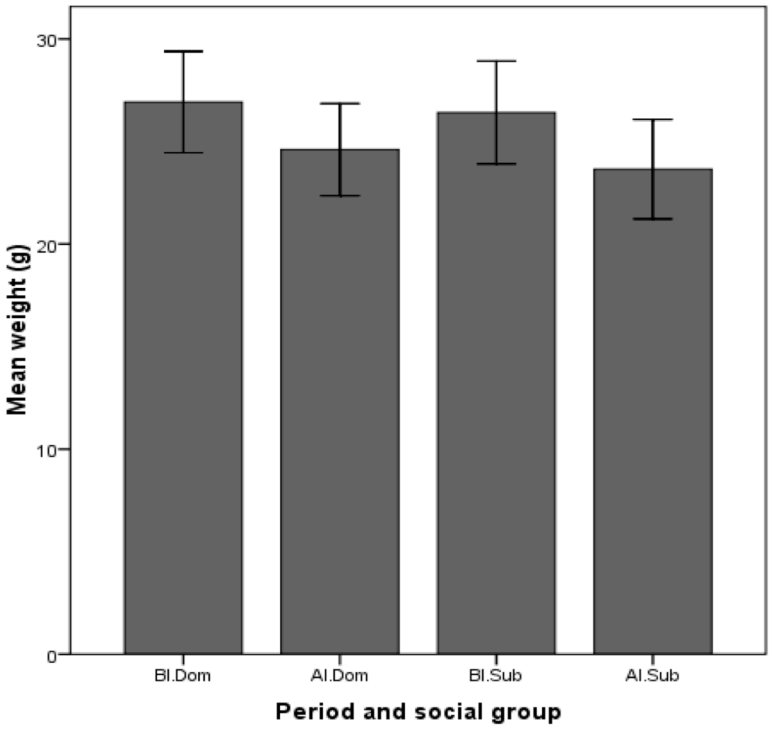

Figure 4. Mean weight (± SEM) of dominant and subordinate fish before and after the social interaction. BI.Dom: dominant - before the interaction; AI.Dom: dominant – after the interaction; BI.Sub: Subordinate – before the interaction; AI.Sub: Subordinate - after the interaction.

Table 1. Summary of time before first acceptance of food and feeding latency score (*i.e.* feeding response score) during the isolation period, and weight of competing individuals before the interaction and 1, 7 and 14 days after the first interaction.

Fish Code No.	Initial Weight (g)		Time before first food acceptance (hr)		Latency score		Weight one day after first interaction (g)		Weight seven days after first interaction (g)		Weight 14 days after first interaction (g)	
14 vs 1	24.4	23.9	2.39	3.51	21	22	25.1	23.4	30.5	25.5	35.1	28.0
3 vs 15	23.1	23.1	0.73	1.74	25	23	25.5	22.9	29.4	25.5	31.3	27.3
5 vs 18	25.1	25.0	2.47	3.44	23	23	25.3	24.2	26.2	27.4	30.7	28.3
9 vs 7	19.9	20.1	1.70	5.00	23	13	21.8	22.1	28.1	25.1	29.6	27.2
32 vs 8	22.6	21.5	0.16	3.73	21	19	23.1	20.5	25.9	23.1	28.5	24.9
11 vs 6	28.4	29.6	1.47	6.80	22	22	28.8	30.5	31.9	31.6	33.9	33.5
13 vs 4	24,.8	24.6	2.60	3.19	20	24	25.4	25.0	29.3	30.4	32.2	33.0
16 vs 2	28.0	27.6	0.34	8.19	22	22	28.3	26.5	38.3	30.0	40.2	30.7
45 vs 17	22.0	23.4	0.13	2.37	23	23	22.0	23.1	24.6	28.0	27.5	31.2
10 vs 12	26.2	26.4	2.02	2.98	27	20	27.2	26.8	32.5	31.5	33.9	
59 vs 46	23.3	23.2	0.04	0.18	23	24	23.2	23.6	26.7	24.8	30.8	28.8
33 vs 25	21.9	21.3	0.22	3.96	22	13	21.7	21.5	23.6	24.7	25.9	26.8
38 vs 23	20.0	20.4	0.18	1.40	21	19	19.4	20.5	22.6	24.4	25.9	27.0
40 vs 47	21.8	21.6	0.11	1.20	22	22	21.3	21.2	22.3	23.0	24.9	25.2
29 vs 48	25.8	26.3	0.17	0.65	25	25	26.4	25.8	29.2	30.5	31.9	33.1
37 vs 34	22.8	23.1	0.16	0.24	19	20	24.4	22.8	27.2	23.9	32.8	25.6
26 vs 35	25.5	25.4	0.07	3.71	23	14	25.8	25.1	27.4	27.2	29.5	30.8
24 vs 27	24.7	24.7	0.09	0.20	22	20	26.7	23.6		27.9		31.1
28 vs 36	24.0	24.1	0.02	0.24	27	17	23.3	23.0	26.5	27.6	28.5	33.0
49 vs 22	31.6	30.5	0.22	0.30	21	21	32.3	32.3	34.7	34.7	36.8	35.4
53 vs 21	28.1	28.2	0.13	3.10	14	23	27.1	28.7	30.0	31.6	31.8	35.1
43 vs 31	25.2	25.4	0.22	1.14	20	19	25.1	25.7	27.4	28.8	29.9	30.2
50 vs 30	25.5	25.5	0.02	0.41	19	20	25.1	26.0	27.0	28.6	29.4	30.8
44 vs 19	28.3	29.3	0.19	0.74	18	19	29.9	28.4	35.0	32.1	40.2	34.3
58 vs 39	26.5	20.9	0.06	0.30	24	20	26.4	21.0	28.8	23.6	31.6	27.0
57 vs 54	24.9	24.8	0.03	0.85	25	17	24.1	23.8	26.3		28.6	
51 vs 41	22.2	22.4	0.09	3.12	25	23	21.5	22.5	23.2	23.5	26.4	26.5
42 vs 56	27.7	27.2	0.10	0.30	22	18	27.0	26.4	28.3	27.0	30.3	29.5
52 vs 55	24.3	24.6	0.18	0.30	28	13	23.7	23.7	25.1	25.1	25.5	28.7

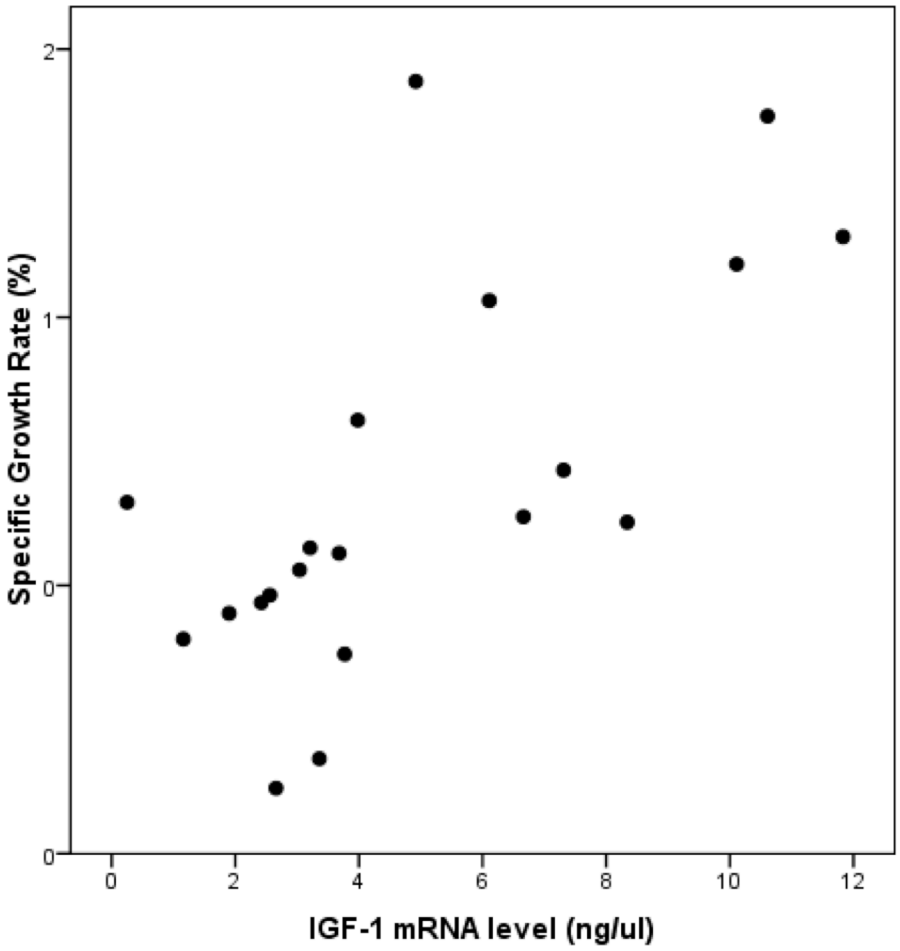

Figure 5. Correlation between IGF-I mRNA level (ng/µl) and specific growth rate of the dominant and subordinate fish, n = 20, $r = 0.60$, $P < 0.01$

Table 2. Average size of the eggs collected in each treatment. LSR, low stress response group; LHR, high stress response group

TREATMENT (SOCIAL GROUP)	AVERAGE SIZE OF THE EGGS (mm)			MEAN± SD [1, 2]
	REPLICATION			
	1	2	3	
LSRHSR♂♀	2.44	2.42	2.52	2.46[b] ± 0.0529
LSR♂♀	2.91	2.79	2.87	2.86[a] ± 0.0611
HSR♂♀		2.16		2.16[c]
LSR♂HSR♀	2.31	2.29	2.23	2.21[c] ± 0.0862
HSR♂LSR♀	2.62	2.54	2.50	2.55[b]± 0.0611

[1]Analysis based on unequal number of replications
[2]Means with similar superscript letters are not significantly different at 5 % level of significance by LSD

Table 3. Sperm motility scoring for the respective accumulated feeding response score by the male breeders.

FEEDING RESPONSE SCORE	MOTILITY SCORE
0	3
1	5
2	4
5	5
6	10
8	10
10	10
11	10
12	10
14	10
15	10
18	10

Table 4. Sperm density (sperm per ml) of the breeders under the accumulated feeding response score

FEEDING RESPONSE SCORE	SPERM DENSITY (SPERM PER ML)
0	$2.025 \times 10^{9(i)}$
1	$3.100 \times 10^{9(h)}$
2	$3.125 \times 10^{9(h)}$
5	$3.750 \times 10^{9(g)}$
6	$4.200 \times 10^{9(feg)}$
8	$4.075 \times 10^{9(fg)}$
10	$4.375 \times 10^{9(fe)}$
11	$4.725 \times 10^{9(de)}$
12	$5.100 \times 10^{9(cd)}$
14	$5.425 \times 10^{9(c)}$
15	$9.400 \times 10^{9(b)}$
18	$1.063 \times 10^{10(a)}$

Means with similar superscript letters are not significantly different at 5 % level of significance CV = 49.62%

Table 5. Spawning success during the 2nd production cycle.

TREATMENT (SOCIAL GROUP)	SPAWNING SUCCESS DURING THE 2ND PRODUCTION CYCLE			MEAN ± SD[1, 2]
	REPLICATION			
	1	2	3	
LSRHSR♂♀	33.33	50.00	66.67	50.00[a] ± 16.67
LSR♂♀	33.33	16.67	33.33	27.78[a] ± 9.62
HSR♂♀	0	0	0	0.00[b] ± 0.00
LSR♂HSR♀	66.67	33.33	50.00	50.00[a] ± 16.67
HSR♂LSR♀	33.33	33.33	16.67	27.78[a] ± 9.62

[1] Based on arcsine square root percentage transformation
[2] Means with similar superscript letters are not significantly different at 5% level of significance

Table 6. Average eggs produced per female at one spawning.

TREATMENT (SOCIAL GROUP)	AVERAGE EGGS PRODUCED PER FEMALE			MEAN ± SD[1, 2]
	REPLICATION			
	1	2	3	
LSRHSR♂♀	711	597	608	638.67[bc] ± 62.88
LSR♂♀	813	1113	929	951.67[a] ± 151.28
HSR♂♀	0	0	0	0.00[d]
LSR♂HSR♀	632	417.5	436	495.17[c] ±118.86
HSR♂LSR♀	730	946	715	797.00[ab] ± 129.26

[1] Analysis based on log (x+1) transformation
[2] Means with similar superscript letters are not significantly different at 5% level of significance

Table 7. Total number of eggs produced in the 1st and 2nd production cycles.

TREATMENT (SOCIAL GROUP)	NUMBER OF EGGS PRODUCED DURING THE 1ST AND 2ND CYCLES							
	1ST PRODUCTION[1]			MEAN[3] (± SD)	2ND PRODUCTION[2]			MEAN[3] (± SD)
	R1	R2	R3		R1	R2	R3	
LSRHSR♂♀	2551	2421	4809	3260.33[a] (± 1342.76)	1423	1793	2435	1883.67[a] (± 512.06)
LSR♂♀	3290	2016	2147	2484.33[a] (± 700.80)	1626	1113	1858	1532.33[a] (± 381.23)
HSR♂♀		2142		2142.00[a]	0	0	0	0.00[b]
LSR♂HSR♀	609	704	1289	867.33[b] (± 368.25)	2528	835	1309	1557.33[a] (± 873.39)
HSR♂LSR♀	1073	506	873	817.33[b] (± 287.57)	1459	1892	715	1355.33[a] (± 595.31)

[1] Analysis based on unequal number of replications
[2] Analysis based on log (x+1) transformation
[3] Means with similar superscript letters are not significantly different at 5 % level of significance

Table 8. Total egg production in the two production cycles.

TREATMENT (SOCIAL GROUP)	TOTAL EGG PRODUCTION		
	1ST PRODUCTION	2ND PRODUCTION	TOTAL EGG PRODUCED
	MEAN ± SD	MEAN ± SD	
LSRHSR♂♀	3260.33[a] ± 1342.76	1883.67[a] ± 512.06	5144.00[a]
LSR♂♀	2484.33[a] ± 700.80	1532.33[a] ± 381.23	4016.66[ab]
HSR♂♀	2142.00[a]	0.00[b]	2142.00[c]
LSR♂HSR♀	867.33[b] ± 368.25	1557.33[a] ± 873.39	2424.66[bc]
HSR♂LSR♀	817.33[b] ± 287.57	1355.33[a] ± 595.31	2172.66[bc]

Means with similar superscript letters are not significantly different at 5% level of significance

Table 9. Hatching rate of the collected eggs over two production cycles.

TREATMENT (SOCIAL GROUP)	HATCHING RATE IN TWO CYCLES (%)							
	1ST PRODUCTION[1]			MEAN ± SD	2ND PRODUCTION[2]			MEAN ± SD[3]
	R1	R2	R3		R1	R2	R3	
LSRHSR♂♀	83.73	82.94	68.12	78.26[a] ± 8.79	87.7	86.11	76.88	83.56[a] ± 5.84
LSR♂♀	93.50	93.70	90.13	92.44[a] ± 2.01	84.56	81.49	86.33	84.13[a] ± 2.45
HSR♂♀	0.00	76.00	0.00	76.00[a]	0	0.00	0.00	0.00[b] ± 0.00
LSR♂HSR♀	80.62	88.78	93.64	87.68[a] ± 6.58	85.88	90.18	77.23	84.43[a] ± 6.60
HSR♂LSR♀	87.51	86.76	69.53	81.27[a] ± 10.17	78.48	79.33	84.34	80.72[a] ± 3.17

[1] Analysis based on unequal number of replications
[2] Analysis based on arcsine square root percentage transformation
[3] Means with similar superscript letters are not significantly different at 5 % level of significance

Table 10. Survival rate of the post-fry after three weeks.

TREATMENT (SOCIAL GROUP)	SURVIVAL RATE (%) IN TWO CYCLES							
	1ST PRODUCTION[1]			MEAN[3/] (± SD)	2ND PRODUCTION[2]			MEAN[3] (± SD)
	R1	R2	R3		R1	R2	R3	
LSRHSR♂♀	71.15	75.34	49.32	65.27[a] (± 13.97)	72.17	81.82	61.93	72.00[a] (± 9.95)
LSR♂♀	75.93	87.50	82.07	81.83[a] (± 5.79)	72.88	75.56	80.52	78.14[a] (± 3.88)
HSR♂♀		47.53		47.53[b]	0	0	0	00.00[c] (± 0.00)
LSR♂HSR♀	55.5	68.47	69.98	64.65[a] (± 7.96)	64.44	78.8	63.41	68.26[ab] (± 8.60)
HSR♂LSR♀	68.59	55.53	52.58	58.90[b] (± 8.52)	58.67	59.73	55.52	57.97[b] (± 2.19)

[1] Analysis based on unequal number of replications
[2] Analysis based on arcsine square root percentage transformation
[3] Means with similar superscript letters are not significantly different at 5 % level of significance

Table 11. Length of the post-fry after three weeks rearing period

LENGTH OF THE POST-FRY AFTER 3 WEEKS (mm)								
TREATMENT (SOCIAL GROUP)	1st PRODUCTION[1]			MEAN ± SD[3]	2nd PRODUCTION[2]			MEAN ± SD[3]
	R1	R2	R3		R1	R2	R3	
LSRHSR♂♀	18.57	19.01	19.37	18.98^{b} (± 0.40)	18.61	18.17	18.33	18.37^{c} (± 0.22)
LSR♂♀	21.18	20.88	20.66	20.91^{a} (± 0.26)	20.67	19.79	20.56	20.34^{a} (± 0.48)
HSR♂♀	0.00	13.88	0.00	13.88^{c}	0.00	0.00	0.00	0.00^{d} (± 0.00)
LSR♂HSR♀	18.01	18.81	18.14	18.32^{b} (± 0.43)	18.56	18.10	18.22	18.29^{c} (± 0.24)
HSR♂LSR♀	19.68	19.31	19.14	19.38^{b} (± 0.28)	19.49	19.24	19.00	19.24^{b} (± 0.25)

[1] Analysis based on unequal number of replications
[2] Analysis based on arcsine square root percentage transformation
[3] Means with similar superscript letters are not significantly different at 5 % level of significance

Table 12. Weight of the post fry after three weeks rearing period.

WEIGHT OF THE POST FRY AFTER 3 WEEKS (g)								
TREATMENT (SOCIAL GROUP)	1st PRODUCTION[1]			MEAN ± SD[3]	2nd PRODUCTION[2]			MEAN ± SD[3]
	R1	R2	R3		R1	R2	R3	
LSRHSR♂♀	0.26	0.26	0.27	0.26^{b} ± 0.0058	0.24	0.23	0.25	0.24^{c} ± 0.0100
LSR♂♀	0.33	0.30	0.30	0.31^{a} ± 0.0173	0.32	0.29	0.30	0.30^{a} ± 0.0153
HSR♂♀	0.00	0.11	0.00	0.11^{d}	0.00	0.00	0.00	0.00^{d} ± 0.0000
LSR♂HSR♀	0.23	0.24	0.24	0.24^{c} ± 0.0058	0.24	0.23	0.23	0.23^{c} ± 0.0058
HSR♂LSR♀	0.29	0.28	0.28	0.28^{b} ± 0.0058	0.28	0.27	0.27	0.27^{b} ± 0.0058

[1] Analysis based on unequal number of replications
[2] Analysis based on log (x+1) transformation
[3] Means with similar superscript letters are not significantly different at 5 % level of significance

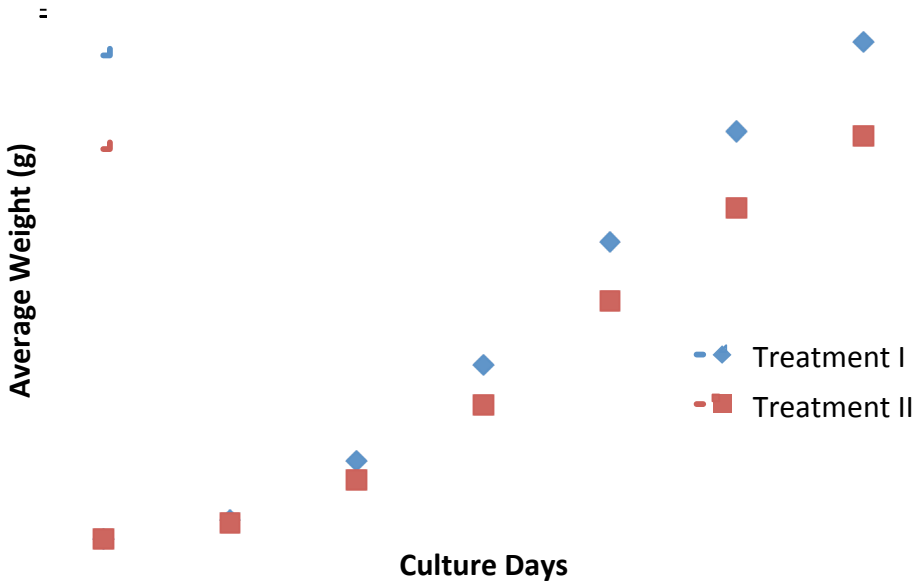

Figure 6. Average weight of Nile tilapia during 90 days of culture in ponds. Treatment I – fingerlings derived from low stress response broodfish; Treatment II - fingerlings derived from high stress response broodfish.

Table 13. Growth performance of Nile tilapia produced from broodstock with low and high stress responses.

Parameters	**Treatments**	
	Sex-reversed fingerlings produced from Low Stress Response Broodfish (Mean ± SEM)	**Sex-reversed fingerlings produced from High Stress Response Broodfish** (Mean ± SEM)
Initial weight (g)	0.199 ± 0.10	0.212 ± 0.20
Final average weight (g)	111.27 ± 19.20	90.31 ± 24.10
Initial length (cm)	2.93 ± 1.20	2.90 ± 2.00
Final average length (cm)	17.16 ± 0.77	17.44 ± 2.60
Gain in weight (g)	111.07 ± 19.10	90.10 ± 23.90
Daily gain in weight (g)	1.23 ± 0.20	1.00 ± 0.30
Gain in length (cm)	14.2 ± 0.80	14.5 ± 0.70
Daily gain in length (cm)	0.158 ± 0.01	0.162 ± 0.01
Feed Conversion Ratio	1.4 ± 0.08	1.7 ± 0.23
Specific Growth Rate (%)	5.51 ± 0.33	5.66 ± 0.55
Yield per hectare (kg ha^{-1})	1596.7 ± 219.0	1221.7 ± 407.0
Feed consumed per hectare (kg ha^{-1})	2205.3 ± 326.30	1890.2 ± 371.50
Survival (%)	73.0 ± 3.90	72.3 ± 5.20

Table 14. Average minimum and maximum readings for water quality parameters during the culture period.

Parameters	Sex-reversed fingerlings produced from Low Stress Response Broodfish		Sex-reversed fingerlings produced from High Stress Response Broodfish	
	Min	Max	Min	Max
Dissolved Oxygen (mg-L^{-1})	2.4	4.2	1.8	4.1
Water Temperature (^{o}C)	28.2	30.1	28.1	30.0
Hydrogen-Ion (pH)	7.3	9.0	7.3	9.1
Total Ammonia Nitrogen (mg L^{-1})	0.051	0.162	0.054	0.190
Secchi Disc Visibility (cm)	20	60	21.7	56.7
Alkalinity (mg L^{-1})	107.7	159.0	101.0	151.3
Phosphorus (mg L^{-1})	0.092	0.237	0.083	0.247

Table 15. Body weight and survival of Nile tilapia reared at different stocking densities in hapas.

Treatment	Stocking Density (pcs)	nitial Weight g)	Final Weight (g)	al
1	250	$7.30^{a} \pm 1.69$	$8.13^{x} \pm 2.05$	$87.6^{a} \pm 9.59$
2	250	$4.15^{b} \pm 0.16$	$6.75^{xy} \pm 0.33$	$61.10^{b} \pm 15.49$
3	500	$4.87^{b} \pm 0.77$	$4.94^{z} \pm 0.25$	$70.05^{b} \pm 4.37$
4	1000	$4.87^{b} \pm 0.77$	$4.66^{z} \pm 0.18$	$67.00^{b} \pm 4.61$

Values represent mean ± standard deviation. 1 (control) - Low density, mixed-sex; 2 - Low density, sex-reversed; 3 - Medium density, sex-reversed; and 4 - High density, sex-reversed. Data with the same letter superscripts are not significantly different.

Table 16. Specific growth rates (%) of Nile tilapia reared at different stocking densities during the course of the study

Treatment	Day 0-10	Day 10-24	Day 24-30	Day 0-30
1	-0.039 ± 1.14	1.047[ab] ± 0.91	-0.631 ± 7.98	0.349 ± 1.37
2	1.449 ± 3.03	2.344[a] ± 0.91	0.299 ± 1.75	1.637 ± 1.52
3	0.158 ± 0.92	0.503[b] ± 0.72	-0.135 ± 1.52	0.260 ± 0.27
4	-0.473 ± 1.55	-0.084[b] ± 0.33	0.382 ± 0.53	-0.120 ± 0.53

Values represent percentage mean ± standard deviation on the specific growth rates of Nile tilapia reared at different stocking densities. 1 (control) - Low density, mixed-sex; 2 - Low density, sex-reversed; 3 - Medium density, sex-reversed; and 4 - High density, sex-reversed. Data with the same letter superscripts are not significantly different.

Table 17. Mean IGF-1 mRNA concentration (ng/µl) of Nile tilapia after 7 days of rearing at different stocking densities.

Treatment Density Group	IGF-1 mRNA (ng/µl)
1	27.03[ab] ±5.24
2	31.59[a] ±6.94
3	14.44[b] ±10.90
4	7.79[b] ±7.64

GF-1 mRNA levels (ng cDNA/µl total RNA) values are shown as mean ± standard deviation. Treatment groups: 1 (control) – low density, mixed-sex; 2 - low density, sex-reversed; 3 - medium density, sex-reversed; and 4 - high density, sex-reversed. Data with the same letter superscripts are not significantly different.

Table 18. The hepatosomatic and cholecystic indices of Nile tilapia reared in different stocking densities.

Treatment Density	Liver Size and Hepatosomatic Index			Gall Bladder Size and Cholecystic Index		
	Weight (g)	Length (mm)	HSI (%)	Weight (g)	Length (mm)	CI (%)
1	0.20[a]±0.02	3.55[a]±0.49	1.09[a]±0.31	0.05[a] ±0.01	0.96[a]±0.43	26.4[a]±17.39
2	0.11[b]±0.02	2.17[b]±0.45	1.11[a]±0.20	0.04[ab]±0.01	0.90[a]±0.23	31.5[a]±23.22
3	0.08[c]±0.01	1.70[b]±0.28	0.96[a] ±0.22	0.02[b]±0.004	0.67[a]±0.08	33.37[a]±21.23
4	0.07[c]±0.01	2.07[b]±0.45	0.99[a] ±0.07	0.03[b]±0.004	0.68[a]±0.05	41.3[a]±17.42

Hepatosomal and cholecystic parameters shown as mean ± standard deviation. 1 (control) - low density, mixed-sex; 2 - low density, sex-reversed; 3 - medium density, sex-reversed; and 4 - high density, sex-reversed. Data with the same letter superscripts are not significantly different.

Table 19. Mean blood glucose levels of Nile tilapia reared at different stocking densities

Treatment Density	Blood Glucose (mg/dl)
1	$81.67^{ab} \pm 11.05$
2	$60.67^{bc} \pm 16.01$
3	$94.50^{a} \pm 19.01$
4	$45.75^{c} \pm 15.09$

Blood glucose values are expressed as mean ± standard deviation. 1 (control) - low density, mixed-sex; 2 - low density, sex- reversed; 3 - medium density, sex-reversed; and 4 - high density, sex-reversed. Data with the same letter superscripts are not significantly different.

Table 20. Total red blood cell and white blood cell counts (x 10^9 cells per ml of blood) of Nile tilapia reared at different stocking densities.

Treatment Density	RBC	WBC
1	3.7^{a}	1.576^{a}
2	2.85^{b}	1.573^{a}
3	2.45^{b}	1.125^{b}
4	2.0^{c}	0.496^{c}

Treatment groups: 1 (control) - low density mixed-sexed; 2 - low density sex-reversed; 3 - medium density, sex-reversed; and 4 - high density sex-reversed. Values with the same letter superscripts are not significantly different.