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Aggression and Mortality among Nile Tilapia (*Oreochromis niloticus*) Maintained in the Laboratory at Different Densities

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Abstract: Because Nile tilapia (*Oreochromis niloticus*) density may affect stress levels, metabolism and survival among tilapia in laboratory experimental studies and thus may affect experimental results, tilapia were held in a commonly-used commercial tank system for 30 days to assess the relationship between fish density and behavior patterns, percent cumulative survival and blood glucose levels. Fish (15.34±0.34 g) were placed in tanks with 7 L (7L group) of water at 3 different densities, 5 (7L-5; 11.0 kg m^{-3}), 10 (7L-10; 21.9 kg m^{-3}), or 15 (7L-15; 32.9 kg m^{-3}) fish per tank, or with 19 L (19L group) water at 3 different densities, 5 (19L-5; 4.0 kg m^{-3}), 10 (19L-10; 8.1 kg m^{-3}), or 15 (19L-15; 12.1 kg m^{-3}) fish per tank. General patterns of behavior involving dominant and subordinate fish were observed: Biting was correlated with poor fin and body condition, ramming and mouth-fighting, while ramming was correlated with mouth-fighting. Mortalities were negatively associated with light coloration. Mortalities in all of the 7 L tanks began one day after fish were placed in the tanks and often continued for 21-28 days or until only one fish remained in the tank and mortalities in the 19L tanks began 3 days after fish were placed in the tanks. Percent cumulative survival was highest among the 19L-10 and 15 (100-93%) tanks in the 19L groups and the 7L-5 and 10 (53%) tanks in the 7L groups over the 30-day study period. Percent cumulative survival in the 7L-10 or 15 tanks was significantly lower than in 19L-10 and 15 tanks ($p<0.0144$ and $p<0.0001$, respectively). The 19L-15 and 7L-5 tanks had approximately the same weight per volume density, but percent cumulative survival between the 2 groups was significantly different ($p<0.0173$). Blood glucose levels of fish in the 7 L tanks increased significantly over 48 h and then decreased to baseline levels again 24 h later. The findings in this study indicate that while Nile tilapia density can influence percent cumulative survival and blood glucose levels in a tank, the physical size and dimensions of the tank and water volume may also affect these values. Based on our research with two commercial aquaria and tank systems commonly used in laboratories, 15 g Nile tilapia could be held at densities of 11.0-21.9 kg m^{-3} in 7 L tanks but preferentially maintained at densities of 8.1-12.1 kg m^{-3} in 19 L tanks to reduce mortality from aggression and ensure proper fish welfare.

Key words: Fish welfare, Nile Tilapia, aquaculture, aggression, stress, social hierarchy, glucose

INTRODUCTION

Nile tilapia (*Oreochromis niloticus*) housed in laboratory aquaria often experience overcrowding. Fish in overcrowded environments may be increasingly subject to acute or chronic stressors, such as aggressive interactions (Gonçalves, 1993; Barcellos *et al*., 1999) and suboptimal water quality (Barton and Iwama, 1991). Such stressors can cause decreased weight gain, decreased disease resistance and increased mortalities (Barton *et al*., 1987; Barcellos *et al*., 1999). Nile tilapia commonly establish social hierarchies in fixed spaces and territories and hierarchies among fish may be established based on size, sex, residency, previous hierarchical rank, social experience and reproductive stage (Francis, 1983; Beacham, 1987; Halperin and Dunham, 1994; Giaquinto and Volpato, 1997).

Social status includes dominant and subordinate fish and the dominant fish may have better access to food (Corrêa *et al*., 2003), territory (Amorim *et al*., 2003) and

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mates (Amorim and Almada, 2005). Subordinate fish often have increased blood cortisol and glucose levels (Zayan, 1991; Pottinger and Pickering, 1992; Balm *et al.*, 1994), metabolic rates (Volpato *et al.*, 1989; Fernandes and Volpato, 1993; Sloman *et al.*, 2000), decreased growth (Fernandes and Volpato, 1993; Metcalfe *et al.*, 1995), immunosuppression (Pottinger and Pickering, 1992; Ortuño *et al.*, 2001) and increased mortality (Pickering, 1993). Aggressive behavior is required to establish and maintain hierarchies among fish and the degree of aggressive behavior may be influenced by social factors such as fish density and physical environmental factors such as aquarium size.

Nile tilapia are commonly used for nutritional, physiological, reproductive, infectious diseases and vaccine laboratory research worldwide. Concrete knowledge of optimal laboratory housing conditions and proper fish welfare for Nile tilapia is still obscure in several aspects, especially the effects of high fish holding densities and behavior in experimental research. Because Nile tilapia density may affect stress levels, metabolism and survival among tilapia in experimental studies and thus may affect experimental results, this study was undertaken to assess the relationship between Nile tilapia density and aquarium size, water quality, behavior patterns, Percent Cumulative Survival (PCS) and, blood glucose levels.

MATERIALS AND METHODS

Fish maintenance: A mixed-sex Nile tilapia (*O. niloticus*; Auburn strain) population with a mean weight of 15.34±0.34 g at the beginning of the study were housed at the USDA-ARS Aquatic Animal Health Research Laboratory (AAHRL) in Chestertown, MD, USA. The fish were maintained on a 12 h: 12 h light: Dark period and were fed daily to satiation with Aquamax Grower 400 (Brentwood, MO, USA). Fish were maintained and handled according to Institutional Animal Care and Use Committee (IACUC)-approved guidelines. Experimental fish were held in 57 L tanks in approximately 30°C water at the AAHRL for over one year before start of the experiment. At the start of the experiment, fish were arbitrarily assigned and moved into 1 of 2 commonly-used commercial aquaria and tank systems. One group of fish was placed in tanks (Aquaneering, San Diego, CA, USA; dimensions in centimeters: 33.0 L×20.3 W×20.3 H) containing 7 L (7L group) of water at 3 different densities: 5 (7L-5; 11.0 kg m^{-3}), 10 (7L-10; 21.9 kg m^{-3}), or 15 (7L-15; 32.9 kg m^{-3}) fish per tank (n = 90) and each density had triplicate tanks. For comparison, another group of fish was held at lower densities in larger tanks (Aquatic Eco-Systems, Apopka, FL, USA; dimensions in centimeters: 40.6 L×20.3 W×25.4 H) containing 19 L (19L group) water at 3 different densities: 5 (19L-5; 4.0 kg m^{-3}), 10 (19L-10; 8.1 kg m^{-3}), or 15 (19L-15; 12.1 kg m^{-3}) fish per tank (n = 30). No replicates of these tanks were included.

The flow-through tanks were supplied with dechlorinated city water. Water temperature, Dissolved Oxygen (DO) and Total Ammonia-Nitrogen (TAN) were measured daily and pH was measured weekly using a YSI 85 m (Yellow Spring Meter, Yellow Springs, OH, USA) and a Fresh Water Aquaculture Kit Model AG-2 (LaMotte, Chestertown, MD, USA) or HACH test kit (Hach Company, Loveland, CO, USA). The overall mean water quality conditions throughout the duration of the experiment were as follows: temperature, 29.82±0.03°C; DO, 4.36±0.04 mg L^{-1}; TAN, 0.29±0.02 mg L^{-1}. Analysis of mean water quality data between tank densities within a group and between groups indicated: the 19L-15 tanks had higher mean temperature levels (30.0°C) than the 19L-5 (29.6°C) and 19L-10 (29.7°C) tanks; The 19L-15 tanks had higher DO levels (4.64 mg L^{-1}) than the 19L-5 (4.13 mg L^{-1}) and 19L-10 (4.12 mg L^{-1}) tanks and 7L-5 tanks had lower TAN levels (0.17 mg L^{-1}) than those of the 7L-10 (0.40 mg L^{-1}) and 7L-15 (0.34 mg L^{-1}) tanks. Where significant differences were noted, no correlations could be drawn between differences in water quality among groups and differences in behavior, survival, or blood glucose. All other water quality parameters within the 7 or 19L tank groups or between corresponding 7 and 19L tanks were not significantly different.

Behavioral observations: Fish behavior in each of the tanks was assessed daily to determine behavior within each category: location in aquaria (mid-to-upper tank, tank bottom, or water surface), swimming patterns (active, stationary, or head-up swimming), feeding patterns (rapid, slow, or no response to food), activity and excitability (active, hyperactive, lethargic, or unresponsive), fin and body condition (normal or poor fin and body condition), body coloration (normal, light, or dark), respiration pattern (active, rapid, or gasping) and acts of aggression (none, biting, ramming, mouth-fighting, cornering, or chasing). Behavior patterns were assessed daily by counting fish in each tank exhibiting a behavior and acts of aggression were determined by observing each tank for 5 min and counting the total incidents of aggression for 30 days. Mortalities were noted and removed twice daily for 30 days.

Blood glucose measurement: Baseline blood glucose (0 h at the beginning of the study) was measured in 7 fish from fish stocks of corresponding weight and size that were not subsequently placed in the experimental tanks. Three separate, additional 7L-Glucose (G) tanks containing fish at the 5 (7L-5G), 10 (7L-10G), or 15 (7L-15G) fish per tank densities were also maintained for blood glucose sampling. These ancillary tanks were set up in the same manner as the other 7L tanks and were included so that fish monitored for behavioral changes were not removed and handled for bleeding. Two fish (one dark and one light colored fish) from these additional tanks were bled and returned to their tanks at 24, 48, 72, 120 and 168 h after the beginning of the study. Because previous studies have indicated that dominant fish have light coloration and submissive tilapia have dark coloration (Pottinger and Pickering, 1992; Fernandes and Volpato, 1993; Corrêa *et al*., 2003), a dark and a light colored fish were sampled to compare blood glucose between presumptively dominant and submissive fish. Normal coloration among Nile tilapia was considered gray coloration with black stripes. Blood glucose concentrations are considered rapid markers of fish stress responses (Andersen *et al*., 1991; Chen *et al*., 1995; Reubush and Heath, 1996; Cech *et al*., 1996; Ortuño *et al*., 2001; Evans *et al*., 2006) and they were determined using the methods of Evans *et al.* (2003, 2004, 2006). Briefly, blood glucose was measured with a One Touch Ultra Brand Meter and test strips (Lifescan, Milpitas, CA). Caudal vein blood samples were obtained using a tuberculin syringe and 27-gauge needle, a 5-10 μL blood drop was placed onto a clean glass slide, the test strip was dipped in the blood drop, the confirmation window was allowed to completely fill with the blood drop and the blood glucose reading was displayed in mg dL^{-1} in about 5 sec (Diouf *et al*., 2000; Evans *et al*., 2003). The One Touch accuracy in determining tilapia blood glucose has been established by Evans *et al.* (2003, 2004).

Statistics: Data analysis was performed using proc mixed and proc corr statements from SAS System version 8 software (Cary, NC, USA). Data was combined to create 5-day means for ANOVA analysis to meet the assumption of normality a nd to decrease variability. The number of fish at a given time was converted to percent fish so that the treatments were not biased due to the number of fish observed. Body coloration and mortalities were noted from the 7L-G fish, but these observations were not used for statistical analysis of behavior patterns. Differences in all analysis were considered significant at $p<0.05$.

RESULTS

Behavior: When fish were placed in their respective experimental tanks, a general pattern of behaviors was noted in all tanks of all groups. Often several dark fish were found in groups, situated stationary on the tank bottom or water surface and exhibited poor fin and body condition. A smaller number of light colored fish was often found moving actively in the center of the tanks biting, ramming, cornering, or chasing the dark colored fish or biting, ramming and mouth-fighting other light colored fish in the tank. All tanks generally contained 0-7 dark colored fish and 0-2 light fish. Few significant differences in behavior were noted within groups or in corresponding tanks between groups. All of the 7L tanks contained significantly different numbers of dark colored fish than each other and the 7L-5 and 7L-10 tanks had significantly different numbers of dark colored fish than their corresponding 19L tanks. The 19L-10 and 19L-15 tanks contained significantly increased numbers of normal colored fish more than the 19L-5 tanks and their corresponding 7L tanks. Limited correlations ($p<0.05$) and associations ($0.05<p< 0.10$) between different behaviors were detected. Biting was correlated with poor fin and body condition, ramming and mouth-fighting, while ramming was correlated with mouth-fighting. Mortalities were negatively associated with light coloration and several behaviors were positively associated with each other: Dark coloration, biting and chasing; poor fin and body condition, ramming and chasing; and dark coloration, poor fin and body condition, biting and mouth-fighting.

Mortalities: Tilapia mortalities in the replicate 7L tanks began one day after fish were placed in the tanks and

Table 1: Percent Cumulative Survival (PCS) of Nile tilapia (*Oreochromis niloticus*)[1] maintained in different fish weight per water volume densities in 19L and 7L tanks (L × W × H) for 30 days

Group	Number of tanks	Fish weight per water volume (kg m^{-3})	Percent cumulative survival (%)
19L-5	1	4.0	20a
19L-10	1	8.1	100b*
19L-15	1	12.1	93b*
7L-5	3	11.0	53b
7L-10	3	21.9	53b*
7L-15	3	32.9	11a*
7L-G5	1	11.0	20a
7L-G10	1	21.9	80b
7L-G15	1	32.9	7a

[1]Experimental fish were placed in tanks with 7 or 19 L of water as 5, 10, or 15 fish per tank. Mortalities were monitored for 30 days after placement in the tanks. Different letters indicate significant differences ($p<0.05$) between treatments within a group and an asterisk indicates a significant difference between non-bled tank size groups (7L versus 19L) with the same number of fish per tank

Table 2: Mean blood glucose values (mg dL^{-1}) and Percent Cumulative Survival (PCS) of Nile tilapia (*Oreochromis niloticus*)[1] maintained in different fish weight per water volume densities in the 7L-G blood glucose sampling tanks (L × W × H) through 168 h

Hour sampled	Group/Percent Cumulative Survival (PCS)					
	7L-G5	PCS	7L-G10	PCS	7L-G15	PCS
0	41.4±2.2a	100	41.4±2.2a	100	41.4±2.2a	100
24	38.0±2.0a	100	35.5±3.5a	100	55.5±8.5ab	73**
48	110.0±21.0c	100	178.0±24.0d*	100	100.5±16.5bc	73**
72	55.0±2.0ab	100	46.5±0.5a	100	56.0±29.0ab	60**
120	64.0±30.0ab	100	44.0±11.0a	90	115.5±28.5c*	40**
168	47.5±2.5a	100	43.5±15.5a	90	63.5±13.5ab	27**

[1]Experimental fish were placed in tanks with 7 L of water as 5 (7L-G5), 10 (7L-G10), or 15 (7L-G15) fish per tank and blood glucose levels were monitored through 168 h (7 days) after placement in tanks. Data are represented as mean blood glucose levels±S.E. and different letters indicate significant differences (p<0.05) between time intervals within a treatment group. Single asterisks highlight significant differences in glucose between treatment groups within a given time interval. Double asterisks highlight significant differences in PCS between treatment groups within a given time interval

often continued for 21-28 days or until only one fish remained in the tank. Mortalities among the fish in the 19L tanks began 3 days after fish were placed into the tanks. The 19L-5 tanks had 4 mortalities within 8 days and the 19L-10 tanks had no mortalities while, the 19L-15 tanks had one mortality. PCS was highest among the 19L-10 (100%) or 19L-15 (93%) tanks through the 30-day study period (Table 1). PCS in the 7L-10 (53%) or 7L-15 (11%) tanks was significantly lower than in 19L-10 and 15 tanks ($p<0.0144$ and $p<0.0001$, respectively). PCS in tanks with 5 fish-regardless of tank size-was not significantly different ($p<0.2384$). When comparing differences between different size groups in the 7L tanks, PCS in tanks with 5 or 10 fish (53%) was significantly higher ($p<0.0004$ and $p<0.0001$, respectively) than in tanks with 15 fish (11%); no significant difference in PCS was noted between the 7L-5 or 7L-10 tanks. Though the 19L-15 tanks and the 7L-5 tanks had approximately the same fish weight per water volume density, PCS between the two groups was significantly different ($p<0.0173$). Note that the 19L tanks were not replicated and this may have decreased the statistical power. PCS in the 7L-G tanks was not significantly different in the 7L-G5 (20%) and 7L-G15 (7%) tanks ($p<0.4163$), but survival was significantly higher in the 7L-G10 (80%) tank than in the 7L-G5 ($p<0.0242$) and 7L-G15 ($p<0.0001$) tanks. No significant differences were noted between survival in the 7L and the corresponding 7L-G tanks with the same number of fish per tank. Nineteen mortalities total from the different density groups were cultured on TSA blood agar at 30°C for 48 h and these fish contained a limited but mixed population of bacterial growth. Growth was within normal limits of bacteria found in tilapia from the AAHRL laboratory.

Blood glucose: Mean blood glucose was determined from fish in the additional 7L-G tanks that corresponded to densities in the 7L group through 7 days (Table 2). Baseline blood glucose was 41.4±2.2 mg dL^{-1} and did not change significantly in any holding density group 24 h after fish were placed in the tanks. Mean blood glucose from fish in each group then increased significantly at the 48-h sampling point, ranging from 100.5±16.5 (7L-G15) to 178.0±24.0 (7L-G10) mg dL^{-1}. At this point, PCS ranged from 73 (7L-G15 group) to 100% (7L-G5 and 7L-G10 groups) and PCS in the 7L-G15 group was significantly lower than the other 7L-G groups (Table 2). The blood glucose in all groups decreased to baseline levels again at 72 h and the PCS ranged from 60 (7L-G15 group) to 100% (7L-G5 and 7L-G10 groups). The blood glucose among the tanks with 5 or 10 fish did not change significantly for the remaining sampling periods. However, blood glucose among fish from the 7L-G15 tanks increased significantly again at 120 h (115.5±28.5 mg dL^{-1}) and the PCS ranged from 40 (7L-G15 group) to 100% (7L-G5 and 7L-G10 groups). Blood glucose then fell to baseline levels at 168 h and PCS in the 7L-G15 group (27%) was still significantly lower than the other 7L-G groups. The greatest reduction in survival (100-27%) in the 7L-G15 tanks occurred between 0-7 days while the greatest reduction in survival (100-20%) in the 7L- G5 group occurred between 7-30 days. When comparing blood glucose between groups, no significant differences were noted at any time-point except: the 48 h sample for the 7L-G10 tanks (178.0±24.0 mg dL^{-1}) was significantly higher than the other groups and the 120 h sample for the 7L-G15 tanks (115.5±28.5 mg dL^{-1}) was significantly higher than the other groups. No significant differences ($p<0.8209$) were noted when comparing light (72.0±11.6 mg dL^{-1}) versus dark (68.4±10.6 mg dL^{-1}) fish blood glucose over all sampling days. Differences between light and dark fish were not assessed at single sampling points because only one of each color fish were sampled.

DISCUSSION

Behavior pattern changes were noted among Nile tilapia held in all of the tanks and common behaviors included: changes in coloration, changes in fin and body condition, biting and ramming and these activities may be attributed to dominance and subordinate hierarchies established within the aquaria. Dominant tilapia are known to swim freely in the aquarium, chasing or attacking subordinate fish; subordinate tilapia are known to swim slowly, withdraw in the face of challenge by dominant fish, stay at the top or bottom of the water column and exhibit more injuries than dominant fish (Corrêa *et al.*, 2003; Amorim and Almada, 2005). Some correlations in behavior were noted, including an inverse correlation between light coloration and mortalities. Thus, in groups with less light fish coloration, there were more mortalities. Although changes in coloration can be exhibited in some disease processes, there were no overt indications of disease and bacterial cultures were within normal limits. Presumably less light coloration and more normal or dark coloration can be attributed to a social hierarchy in the tank. Generally, submissive tilapia have a darker coloration and dominant fish are lighter (Pottinger and Pickering, 1992; Fernandes and Volpato, 1993; Corrêa *et al.*, 2003) and decreased light fish coloration may represent one dominant fish and more fighting in the tank.

Though, stocking density had an overall influence on PCS, it had limited influence on behavior. Oddly, many of the activities generally associated with aggressive behavior (ramming and mouth-fighting) did not correlate with mortalities, though many of the behaviors were correlated with each other (biting with poor fin and body condition, ramming and mouth-fighting, plus ramming with mouth-fighting). The apparent lack of correlations may be an aspect of the statistical program groupings, which linearized data over 5-day intervals and may not have accounted for daily fluctuations in activity that resulted in mortalities. In addition, this study may also not have observed mortality-associated behaviors or such behaviors were not increasingly displayed during the 5 min observation periods. Further, prevalent confrontational displays and fights present immediately after fish were placed in tanks decrease over time as a social hierarchies are established (Barcellos *et al.*, 1999; Fernandes and Volpato, 1993). Because behavior changes were examined over a 30 day period, a decrease in aggressive behavior over time may have decreased the overall mean behavior changes. Alternatively, it is possible that not all mortalities were related to behavior. The effects of different densities in culture systems have been studied with varying results, irrespective of whether Nile tilapia were studied under laboratory or aquaculture settings. Several authors observed Nile tilapia in different densities and found the stocking density affected growth rate, blood glucose and cortisol and survival (Dambo and Rana, 1992; Barcellos *et al.*, 1999; El-Sayed, 2002; Abdel-Tawwab *et al.*, 2005; Khater, 2006). However, other authors indicate that stocking density did not affect growth rate and/or survival (Macintosh and De Silva, 1984; Hossain *et al.*, 2004). Meanwhile, Ridha (2006) found that growth but not survival was negatively affected by stocking density at approximately 20.0 kg m^{-3}. Abou *et al.* (2007) observed that increased Nile tilapia densities up to 0.08 kg m^{-3} did not affect survival but still increased weight gain and feed conversion. Several of these authors have attributed the detrimental effects of density to social stressors including aggression and cannibalism (Macintosh and De Silva, 1984; Dambo and Rana, 1992; El-Sayed, 2002) and general factors such as higher chronic stress responses, lower weight gain and decreased resistance to other stressors (Barcellos *et al.*, 1999). However, several improvements in husbandry conditions, including nutrition (Macintosh and De Silva, 1984; Abdel-Tawwab *et al.*, 2005; Abou *et al.*, 2007), water quality (Chowdhury *et al.*, 2006; Khater, 2006) and Nile tilapia strain (Ridha, 2006), have been shown to counteract the detrimental effects of density.

In the study presented here, aggressive encounters and dominant/submissive relationships among the Nile tilapia were observed daily. PCS data indicates that both very low and very high Nile tilapia densities may create a social environment that results in decreased survival in aquaria, although the mechanisms for mortality would necessitate further study. The fish in the 4.0 and 32.9 kg m^{-3} tanks had PCS of 20 and 7-11%, respectively. Meanwhile, the larger tanks with the greatest densities of 10 fish (8.1 kg m^{-3}) and 15 fish (12.1 kg m^{-3}) had the significantly highest survival (93-100%) and the smaller tanks with 5 (11.0 kg m^{-3}) and 10 fish (21.9 kg m^{-3}) had moderate PCS (53%). Interestingly, the 7L tank with 5 fish and the 19L tank with 15 fish had approximately the same fish weight per water volume, but the 19L tank had significantly higher PCS. The findings in this study indicate that while Nile tilapia density, the physical size and dimensions of the tank and water volume can influence PCS in a tank, blood glucose may also affect these values.

Balm *et al.* (1994) paired *O. mossambicus* in tanks and observed increased cortisol in one fish out of the pair after 3 h. Since, blood samples in this study were taken at 24 h, an acute significant increase in blood glucose would have been missed. However, the data in this study indicates that Nile tilapia experience significant stress relatively later (48 h) after placement with other fish. Fernandes and Volpato (1993) paired Nile tilapia and found that subordinate fish blood glucose was significantly increased over those of dominant fish 48 h after pairing, though no significant differences were noted after 96 h. Stress levels may decrease to normal levels once a social hierarchy has been established (Barcellos *et al.*, 1999), also helping to explain why no differences in glucose were observed between dark and light fish over the course of the study. Several authors have also observed acute stress responses that resolve within 24 h with stressor removal (Strange, 1980; Evans *et al.,* 2003) though the stressor was not removed in this study. In this study, the decrease of blood glucose, presumptive stress resolution and creation of social hierarchy appears to have occurred within 72 h in the tanks with 5 or 10 fish and within 168 h in the tanks with 15 fish.

Correa *et al.* (2003) also paired Nile tilapia in a single tank and then obtained samples for blood glucose and cortisol. They determined that stress levels were increased in both paired fish over those of isolated fish, but there were no significant differences between blood glucose and cortisol of dominant and subordinate fish. As in the study here, Correa *et al.* (2003) observed that dominance or subordination among Nile tilapia did not correspond to stress level. Several other authors have suggested that stress responses are more significant in subordinate fish than dominant fish (Zayan, 1991; Fernandes and Volpato, 1993; Balm *et al.*, 1994). However, social stress may be an important factor for both dominant and subordinate fish during the rapid creation of a social hierarchy, but may impact the subordinate fish more significantly over a longer time period. Correa *et al.* (2003) noted that dominance among 2 fish paired alone was completely determined within 6 h of pairing, though it may have been determined within 2-3 h. Fernandes and Volpato (1993) suggested that Nile tilapia hierarchical social relationships were determined within 10-45 min after pairing. Oliveira and Almada (1998) observed grouped *O. mossambicus* and determined that hierarchical social relationships were determined within a few h of group formation. Though both dominant and subordinate fish may exhibit stress effects during this short period of time, acute stress does not appear to affect survival. However, chronic stress may adversely affect the fish, most likely the subordinate fish.

CONCLUSION

Based on our research with two commercial aquaria and tank systems commonly used in laboratories, 15 g Nile tilapia could be held at densities of 11.0 to 21.9 kg m^{-3} in 7L tanks but preferentially maintained at densities of 8.1-12.1 kg m^{-3} in 19L tanks to reduce mortality from aggression and ensure proper fish welfare.

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REFERENCES

Abdel-Tawwab, M., M.A.A. Mousa, S.M. Sharaf and M.H. Ahmad, 2005. Effect of crowding stress on some physiological functions of Nile tilapia, *Oreochromis niloticus* (L.) fed different protein levels. Int. J. Zool. Res., 1: 41-47.

Abou, Y., E.D. Fiogbé and J.-C. Micha, 2007. Effects of stocking density on growth, yield and profitability of farming Nile tilapia, *Oreochromis niloticus* L., fed *Azolla* diet, in earthen ponds. Aqua. Res., 38: 595-604.

Amorim, M.C.P. and V.C. Almada, 2005. The outcome of male-male encounters affects subsequent sound production during courtship in the cichlid fish *Oreochromis mossambicus*. Anim. Behav., 69: 595-601.

Amorim, M.C.P., P.J. Fonseca and V.C. Almada, 2003. Sound production during courtship and spawning of *Oreochromis mossambicus*: male-female and male-male interactions. J. Fish Biol., 62: 658-672.

Andersen, D.E., S.D. Reid, T.W. Moon and S.F. Perry, 1991. Metabolic effects associated with chronically elevated cortisol in rainbow trout (*Oncorhynchus mykiss*). Can. J. Fish Aquat. Sci., 48: 1811-1817.

Balm, P.H.M., P. Pepels, S. Helfrich, M.L. Hovens and S.E. Wendelaar Bonga, 1994. Adrenocorticotropic hormone in relation to interrenal function during stress in tilapia (*Oreochromis mossambicus*). Gen. Comp. Endocrinol., 96: 347-360.

Barcellos, L.J.G., S. Nicolaiewsky, S.M.G. de Souza and F. Lulhier, 1999. The effects of stocking density and social interaction on acute stress response in Nile tilapia *Oreochromis niloticus* (L.) fingerlings. Aqua. Res., 30: 887-892.

Barton, B.A. and G.K. Iwama, 1991. Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids. Ann. Rev. Fish Dis., 1: 3-26.

Barton, B.A., C.B. Schreck and L.D. Barton, 1987. Effects of chronic cortisol administration and daily acute stress on growth, physiological conditions and stress responses in juvenile rainbow trout. Dis. Aquat. Org., 2: 173-185.

Beacham, J.L., 1987. The relative importance of body size and aggressive experience as determinants of dominance in Pumpkinseed sunfish *Lepomis gibbosus*. Anim. Behav., 36: 621-623.

Cech, J.J., S.D. Bartholow, P.S. Young and T.E. Hopkins, 1996. Striped bass exercise and handling stress in freshwater: Physiological responses to recovery environment. Trans. Am. Fish Soc., 125: 308-320.

Chen, G.R., L.T. Sun, Y.H. Lee and C.F. Chang, 1995. Characteristics of blood in common carp, *Cyprinus carpio*, exposed to low temperatures. J. Appl. Aqua., 5: 21-31.

Chowdhury, M.A.K., Y. Yi, C.K. Lin and E.R. El-Haroun, 2006. Effect of salinity on carrying capacity of adult Nile tilapia *Oreochromis Niloticus* L. in recirculating systems. Aqua. Res., 37: 1627-1635.

Corrêa, S.A., M.O. Fernandes, K.K. Iseki and J.A. Negrão, 2003. Effect of the establishment of dominance relationships on cortisol and other metabolic parameters in Nile tilapia (*Oreochromis niloticus*). Braz. J. Med. Biol. Res., 36: 1725-1731.

Dambo, W.B. and K.J. Rana, 1992. Effects of stocking density on growth and survival of Nile tilapia *Oreochromis niloticus* (L.) fry in the hatchery. Aquac. Fish Manag., 23: 71-80.

Diouf, B., P. Rioux, P. Blier and D. Rajotte, 2000. Use of brook char (*Salvelinus fontinalis*) physiological responses to stress as a teaching exercise. Adv. Physiol. Edu., 23: 18-23.

El-Sayed, A.F.M., 2002. Effects of stocking density and feeding levels on growth and feed efficiency of Nile tilapia (*Oreochromis niloticus* L.) fry. Aqua. Res., 33: 621-626.

Evans, J.J., P.H. Klesius, C.A. Shoemaker and B.T. Fitzpatrick, 2004. *Streptococcus agalactiae* vaccination and infection stress in Nile tilapia, *Oreochromis niloticus*. J. Appl. Aqua., 16: 105-115.

Evans, J.J., D.J. Pasnik, G.C. Brill and P.H. Klesius, 2006. Un-ionized ammonia exposure in Nile tilapia: Toxicity, stress response and susceptibility to *Streptococcus agalactiae*. N. Am. J. Aqua., 68: 23-33.

Evans, J.J., C.A. Shoemaker and P.H. Klesius, 2003. Effects of sublethal dissolved oxygen stress on blood glucose and susceptibility to *Streptococcus agalactiae* in Nile tilapia, *Oreochromis niloticus*. J. Aquat. Anim. Health, 15: 202-208.

Fernandes, M.O. and G.L. Volpato, 1993. Heterogeneous growth in the Nile tilapia: Social stress and carbohydrate metabolism. Physiol. Behav., 54: 319-323.

Francis, R.C., 1983. Experimental effects on agonistic behavior in the paradise fish, *Macropodus opercularis*. Behav., 85: 292-313.

Giaquinto, P.C. and G.L. Volpato, 1997. Chemical communication, aggression and conspecific recognition in the fish Nile tilapia. Physiol. Behav., 62: 1333-1338.

Gonçalves, E., 1993. Estratégias territoriais e reprodutivas da tilápia-do-Nilo (*Oreochromis niloticus*). Master's Dissertation in Zoology, University of São Paulo State, Botucatu.

Halperin, J.R.P. and D.W. Dunham, 1994. Social overstimulation reduces subsequent aggression in *Betta splendens*. Aggress. Behav., 20: 135-142.

Hossain, M.A., R. Roy, S.M. Rahmatullah and A.H.M. Kohinoor, 2004. Effect of stocking density on the growth and survival of GIFT tilapia, *Oreochromis niloticus* fed on formulated diet. Journal of Agriculture and Rural Development Gazipur, 2: 127-133.

Khater, A.M., 2006. Reproductive performance of Nile tilapia (*Oreochromis niloticus*) at different levels of water temperature and rearing of fry at different stocking densities. Egyptian J. Agric. Res., 84: 203-211.

Macintosh, D.J. and S.S. De Silva, 1984. The influence of stocking density and food ration on fry survival and growth in *Oreochromis mossambicus* and *O. Niloticus* female X *O. aureus* male hybrids reared in a closed circulated system. Aquaculture, 41: 345-358.

Metcalfe, N.B., A.C. Taylor and J.E. Thorpe, 1995. Metabolic rate, social status and life-history strategies in Atlantic salmon. Anim. Behav., 49: 431-436.

Oliveira, R.F. and V.C. Almada, 1998. Adrogenization of dominant males in a cichlid fish: Androgens mediate the social modulation of sexually dimorphic traits. Ethol., 104: 841-858.

Ortuño, J., M.A. Esteban and J. Meseguer, 2001. Effects of short-term crowding stress on gilthead seabream (*Sparus aurata* L.) innate immune response. Fish Shellfish. Immunol., 11: 187-197.

Pickering, A.D., 1993. Endocrine-induced pathology in stressed salmonid fish. Fish. Res., 17: 35-50.

Pottinger, T.G. and A.D. Pickering, 1992. The influence of social interaction on the acclimation of rainbow trout. J. Fish Biol., 41: 435-447.

Reubush, K.J. and A.G. Heath, 1996. Metabolic responses to acute handling by fingerling inland and anadromous striped bass. J. Fish Biol., 49: 830-841.

Ridha, M.T., 2006. Comparative study of growth performance of three strains of Nile tilapia, *Oreochromis niloticus*, L. at two stocking densities. Aquac. Res., 37: 172-179.

Sloman, K.A., G. Motherwell, K.I. O'Connor and A.C. Taylor, 2000. The effect of social stress on the Standard Metabolic Rate (SMR) of brown trout, *Salmo trutta*. Fish Physiol. Biochem., 23: 49-53.

Strange, R.J., 1980. Acclimation temperature influences cortisol and glucose concentrations in stressed channel catfish. Trans. Am. Fish Soc., 109: 298-303.

Volpato, G.L., P.M.A. Frioli and M.P. Carrieri, 1989. Heterogeneous growth in fish: some data in the Nile tilapia *Oreochromis niloticus* and a general view about the causal mechanisms. Boletim de Fisiologia Animal, 13: 7-22.

Zayan, R., 1991. The specificity of stress. Behav. Processes, 25: 81-93.