Effects of stocking density on ammonia excretion and the growth of Nile tilapia (*Oreochromis Niloticus* L.)

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Abstract
The effects of stocking density (10, 15, 50 & 75 fish in 65L tank) and ammonia excretion on the growth of Nile tilapia, *Oreochromis niloticus* (12.19 ± 1.21 g) were investigated. Increasing stocking density of Nile tilapia from 15 fish/tank (2.81 g fish/L) to 75 fish/tank (14.07 g fish/L) resulted in associated increase in ammonia level (1.48 ± 0.87 mg/L to 26.44 ± 11.4 mg/L) and significantly lower growth rates. Significantly better feed conversion ratios were found for fish reared at lower (15 fish/tank) stocking densities compared to higher (75 fish/tank) stocking densities. Individual growth rates were significantly better for fish reared at a lower stocking density 15 fish/tank compared to higher stocking density 75 fish/tank and size variation (coefficient of variation in weight) were positively correlated with stocking density. Although water exchange did not have a significant effect on the growth of Nile tilapia for fish stocked at 10 fish/tank (1.88 g fish/L) and 50 fish/tank (9.38 g fish/L), however, the fish in the higher stocking density (9.38 g fish/L) groups and without water exchange, significantly changed the coloration of their bodies (silver to black) which may be due to the lower oxygen levels combined with higher ammonia levels. Ammonia level increased with increasing stocking density and without water exchange. In this study, it may be suggested that when fish reared at higher stocking densities then water exchange must be taken in to consideration so as to help avoid environmental and physiological stress to the fish.

Key words: Nile tilapia, Stocking density, Ammonia excretion, Growth

Introduction
In areas where suitable land and water are limiting for fish culture purposes and the market demand for fish is high, semi-intensive or intensive tank culture systems have been recommended (Sin and Chiu 1983) and found to be economically viable (Liao and Chen 1983). Intensive culture systems generally employ high stocking densities in order to maximise production with minimal water usage. High densities however, have implications for fish welfare. For example, in pond culture of tilapia where water can be stagnant, stocking densities up to 10 fish/m² might be employed (Balarin and Haller 1982). Densities beyond 8 fish/m² have been found to be detrimental to fish due to the build-up of waste metabolites in the pond water (Zohar et al. 1984).
High density culture of tilapia has been shown to be successful (Balarin and Haller 1982), but comparing results with studies conducted on tilapia maintained at lower stocking densities is difficult because individual studies do not address difficulties that arise when there are so many interactive factors involved. Horner et al. (1987) worked with juvenile and adult tilapia and reported that high densities (12 fish/L) fostered faster growth but with slightly greater variance than lower densities (1 fish/L). Stocking density and, therefore, the volume of water per fish is a significant factor in determining optimum production in tank culture systems. Increasing stocking density results in increased ammonia excretion and may lead to stress (Harris et al. 1998) which can result in enhanced energy requirements causing reduced growth rates and utilisation of food (Rusmussen and Korsgaard 1996). While a number of studies have examined growth, survival, and production of various tilapia species under different stocking densities (Suresh and Lin 1992; Siddiqui and Al-Harbi 1995), little information is available on the relationships between water quality such as dissolved oxygen and ammonia excretion with growth performance, stocking density, and size variation (i.e. social hierarchy). The objectives of this study were twofold. First, to assess the effects of stocking density on growth and size variation of Nile tilapia *O. niloticus*. Second, to establish the relationships between stocking density and ammonia excretion by rearing tilapia at high densities with optimal water quality to test the hypothesis that high density would have a detrimental effect on growth but reduce the variance of size.

Materials and methods

Experimental set up

The experiment was conducted between August and November 2001 at the Department of Zoology, University of Aberdeen, Scotland, UK. Nile tilapia (*Oreochromis niloticus* L.) fingerlings of the same age and size (12.19 ±1.21 g) were obtained from the Stirling University Aquaculture Unit, Stirling, Scotland, UK. Prior to the start of the experiments, all fish were reared in the freshwater unit (water temperature 27-30°C) in a large circular stock tank (water volume 150L) at the Department of Zoology, University of Aberdeen, Scotland, UK. Before conducting any experiments, the tanks (65L water volume) were prepared as the tank water passed through a box-type gravel clinoptilite external water filter. The water temperature was kept between 27 and 30°C, and the photoperiod was regulated by a time clock adjusted so that there was 12 hour light and 12 hour darkness.

Food and feeding

The fish were fed a commercial pelleted diet (45% protein, oil 18%, ash 8.5%, fiber 2%; "Ewos" fish food company, UK). The dry weight of the feed was measured following the procedure described in AOAC (1983) and 1 g of wet feed corresponded to 0.9549 g of dry feed. The fish were fed at a ration level of 2.5% body weight once a day and everyday (7 days a week) between 9:00-9:30 h. The ration was adjusted after every sampling
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occasion. The tanks were cleaned daily (where necessary) after one hour of feeding. Three different experiments were conducted to determine the optimum stocking density of juvenile Nile tilapia *O. niloticus* under tank culture condition. The three experiments were as follows:

**Experiment 1:** Effect of stocking density and ammonia excretion on growth of Nile tilapia when oxygen levels are different

**Experiment 2:** Effects of stocking density and ammonia excretion on growth of Nile tilapia when oxygen levels are similar

**Experiment 3:** Changes in individual growth performance and size variation of Nile tilapia under different stocking densities

**Experiment 1:** The effect of stocking density was assessed under 2 varying conditions as uniform water exchange and without water exchange. Here note that, the oxygen levels of different tanks were not similar although oxygen supply was the same. The experiment was carried out between 6 August and 25 August 2001, for a duration of 20 days. Two stocking densities replicated twice with or without water exchange for this experiment. The experimental tanks were designated as: T₁: Lower density (10 fish/65L tank i.e. 1.88 g fish/L) with water exchange daily, T₂: Lower density (10 fish/65L tank i.e. 1.88 g fish/L) with no water exchange, T₃: Higher density (50 fish/65L tank i.e. 9.38 g fish/L) with water exchange daily and T₄: Higher density (50 fish/65L tank i.e. 9.38 g fish/L) with no water exchange.

**Experiment 2:** The effect of stocking density was assessed under 2 varying conditions as uniform water exchange and without water exchange. Here note that, oxygen level was similar in all tanks. This experiment was carried out between 7 September and 25 September 2001, for a duration of 19 days. The experimental protocol for this experiment was same as experiment 1 with the only difference being that the oxygen was maintained at a similar level. This was achieved by more aeration (air stone) being provided in tanks where necessary. The procedures used were the same as for experiment 1 except for the higher density (50 fish/65L tank) tank where additional aeration was provided with more air-stones to keep oxygen level similar with lower stocking density (10 fish/65L tank). The experimental tanks were designated as: T₁: Lower density (10 fish/65L tank i.e. 1.88 g fish/L) with water exchange daily, T₂: Lower density (10 fish/65L tank i.e. 1.88 g fish/L) with no water exchange, T₃: Higher density (50 fish/65L tank i.e. 9.38 g fish/L) with water exchange daily and T₄: Higher density (50 fish/65L tank i.e. 9.38 g fish/L) with no water exchange.

**Experiment 3:** The main objectives of this experiment were to determine the growth variation between individual fish and between fish reared at lower and higher stocking densities. The lower stocking density for this experiment was increased from SD-10 used in experiments 1 and 2 to SD-15 because the fish reared at lower stocking density (SD-10) in experiments 1 and 2 showed aggressive behaviors. Higher stocking density was
used for this experiment i.e. increased from SD-50 (used in experiments 1 and 2) to SD-75 because in experiments 1 and 2 there were no significant differences in growth of Nile tilapia observed between higher and lower stocking densities (SD-10 and SD-50 fish/65L tank, respectively). The increased range between minimum (SD-15) and maximum (SD-75) stocking densities was used to investigate if significant differences in growth could be observed. This experiment was conducted between 4 and 25 November 2001, for a duration of 22 days. Two stocking densities were replicated twice for this experiment. Approximately 30% of the tank water was exchanged daily and oxygen levels were kept similar in all tanks in order to observe the effects of ammonia excretion on growth of Nile tilapia under lower and higher stocking densities. The experimental tanks were designated as: T_1: Lower stocking density (15 fish/65L tank i.e. 2.81 g fish/L) and T_2: Higher stocking density (75 fish/65L tank i.e. 14.07 g fish/L).

Water quality measurements

Water quality parameters were monitored based on daily measurements between 8:00 and 9:00 h in the morning. In tanks where water exchange was necessary, water quality parameters were measured before any exchange of water. Dissolved oxygen (DO, mg/L) was measured using a portable Microprocessor auto cal. DO meter (Model HI 9143, Sensitivity±0.01 mg/L, HANNA Instruments, Portugal). pH was measured with a portable pH meter. For measuring ammonia by using pH meter (Unicam, 9450 pH Meter, Sensitivity ± 0.001/0.01, Cambridge, UK) and ammonia electrode (Model IS 570-NH₃, Sensitivity 57±-2mV, Philips), triplicate water samples from each tank were collected and immediately neutralized by acid and stored at -20°C for later analysis.

Treatments of data and statistical analyses

The equation used to calculate the wet weight specific growth rate (SGR, %/day Ricker 1979). The coefficient of variation of weight (CV_{weight}) was calculated to determine the homogeneity of the size range of fish in each tank. CV_{weight} was obtained according to the following equation: \( CV_{weight} (%) = \frac{SD}{X} \times 100 \); Where, CV_{weight} is the coefficient of variation of mean weight, SD is the standard deviation of mean wet weight and X is the mean wet weight (g) of fish (Gomes et al. 2000). The feed conversion ratio (FCR) was calculated (Suresh and Lin 1992) using the following formula: FCR = total mg dry weight feed fed/g wet weight gain (fish final wet weight in g – initial fish wet weight in g). Differences in growth under different stocking densities, dissolved oxygen and ammonia levels in response to with and without water exchange were analyzed by ANOVA using SPSS 9.0 statistical computer package for Windows 95/98. Where ANOVA showed significant differences between or within the treatments, Scheffe's multiple comparison tests were performed. Data are presented as means with their standard errors (± S.E). A probability level of 5% was considered for significant differences in all tests.
Results

Experiment 1: Effect of stocking density and growth (with and without water exchange) with varying levels of oxygen

There were no significant differences in the mean initial body weights of the four experimental groups of fish used in this experiment (Table 1). By the end of the experiment the groups of fish reared at lower stocking density (SD-10) both with and without water exchange had higher growth rates (SGR, %/day) than fish reared at higher stocking densities (SD-50). Growth rate however, was not significantly different between stocking density or between tanks with and without water exchange (Fig. 1). Feed conversion ratios for the group of fish reared at a lower stocking density were significantly better than those fish at a higher stocking density (Table 1). The variation of size in weight (CV_weight, %) of fish significantly increased with increasing stocking density (Table 1). During the period of experiment 1, dissolved oxygen (DO, mg/L) level significantly decreased with increasing stocking density (Fig. 2b). Levels of ammonia increased significantly with increasing stocking density (Fig. 2a). Here note that, in this experiment the group of fish reared at a higher stocking density (SD-50) and without water exchange exhibited changes in body coloration (from silver to black). In this experiment, it was observed that fish stocked at the lower density (both with and without water exchange) showed better growth rates (not significant) than those reared at a higher stocking density (where oxygen level was lower and ammonia level was higher). The question arises then why is rate of growth of fish at the higher stocking density lower than fish at lower stocking density? Could it be the higher levels of ammonia or due to the lower oxygen level? To answer this question the following experiment was conducted where oxygen levels were kept similar in all tanks.

![Figure 1](image_url)

**Fig. 1.** Comparison of mean initial and final weights (g) in (a) and mean specific growth rates (SGR, %/day) in (b) of fish reared under different stocking densities (SD-10 and SD-50) with and without the water being exchanged during the experimental period (Expt. 1).
Table 1. Mean initial and final weights (g), mean specific growth rates (SGR<sub>m</sub>, %/day), size variations (CV<sub>weight</sub>, %) and mean feed conversion ratios (FCR<sub>m</sub>, mg dry food/g wet weight of fish) of fish reared under different stocking densities (Exp. 1, 2 & 3). Mean values sharing a common superscript (down columns) are not statistically different at the 5% (p < 0.05) level of significance (Scheffe's multiple comparison test).

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Stocking density (fish/65L tank)</th>
<th>Initial weight (g)</th>
<th>Final weight (g)</th>
<th>SGR&lt;sub&gt;m&lt;/sub&gt; (%/day)</th>
<th>CV&lt;sub&gt;weight&lt;/sub&gt; (%)</th>
<th>FCR&lt;sub&gt;m&lt;/sub&gt; (mg/g)</th>
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<td>12.72 ± 0.37&lt;sup&gt;a&lt;/sup&gt;</td>
<td>19.88 ± 3.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.34 ± 0.29&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.38 ± 1.39&lt;sup&gt;a&lt;/sup&gt;</td>
<td>943 ± 174&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>No water exchange</td>
<td>10</td>
<td>12.19 ± 1.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18.53 ± 2.52&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.20 ± 0.73&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.29 ± 1.44&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1019 ± 326&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>50</td>
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<td>18.05 ± 3.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.05 ± 0.50&lt;sup&gt;a&lt;/sup&gt;</td>
<td>19.85 ± 3.51&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1151 ± 361&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>1.34 ± 0.68&lt;sup&gt;a&lt;/sup&gt;</td>
<td>21.46 ± 4.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1176 ± 26&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>36.53 ± 6.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.57 ± 0.42&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>29.55 ± 8.54&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>1.86 ± 1.14&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>1265 ± 366&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>29.19 ± 8.16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.74 ± 0.83&lt;sup&gt;a&lt;/sup&gt;</td>
<td>22.53 ± 7.23&lt;sup&gt;a&lt;/sup&gt;</td>
<td>732 ± 69&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>28.05 ± 5.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.74 ± 0.61&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>12.73 ± 1.39&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>1.86 ± 0.54&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
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<td>75</td>
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<td>21.98 ± 5.41&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>42.70 ± 16.23&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1139 ± 111&lt;sup&gt;b&lt;/sup&gt;</td>
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Effects of stocking density on ammonia excretion of Nile tilapia

Fig. 2. Levels of ammonia (mg/L) in (a) and dissolved oxygen (mg/L) in (b) measured 7 days for fish reared under different stocking densities (SD-10 and SD-50) with and without the water being exchanged during the experimental period (Expt. 1).

Experiment 2: Relationships between stocking density and growth where oxygen levels in the water remain similar

Although no significant differences in growth rates of fish were observed between fish reared at lower (SD-10) and higher (SD-50) stocking densities, there was a general trend of increased growth with decreasing stocking density (Fig. 3). In this experiment dissolved oxygen levels was similar in all tanks (Fig. 4b). Ammonia levels increased significantly with increased stocking density (Fig. 4a). Significantly better FCRs were observed for fish at a lower stocking density and the variation of size in weight (CV_weight, %) of fish significantly increased with increasing stocking density (Table 1). Here it was also found that water exchange did not show significant differences in growth rates of the fish reared at lower or higher stocking densities.

Fig. 3. Comparison of mean initial and final weights (g) in (a) and mean specific growth rates (SGR, %/day) in (b) of fish reared under different stocking densities (SD-10 and SD-50) with and without water being exchanged during the experimental period (Expt. 2).
Experiment 3: Changes in individual growth performance and size variation of Nile tilapia in relation to different stocking densities

From the results of experiments 1 and 2, it was observed that increasing stocking density from 10 fish/65L tank (1.88 g fish/L) to 50 fish/60L tank (9.38 g fish/L), had no significant effect on growth rate. Also exchanging part of the water did not have a significant effect on growth rate. In experiment 3, significantly higher growth rates of fish were observed for fish reared in lower stocking density (SD-15) compared to higher stocking density (SD-75) groups (Fig. 5a & 5b). Significantly better FCRs were observed in fish stocked at a lower density and the variation of size in weight (CV_weight %) of fish significantly increased with increasing stocking density (Table 1). Oxygen levels in the water were similar for all tanks (Fig. 6b) and ammonia levels (Fig. 6a) increased significantly with increased stocking density. Fig. 7 shows the relationships between specific growth rates and ammonia and specific growth rates and stocking density, respectively (data are combined from the above three experiments). There was a significant negative correlation found between specific growth rates and ammonia (Fig. 7).
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Fig. 6. Levels of ammonia (mg/L) in (a) and dissolved oxygen (mg/L) in (b) measured over 22 days for fish reared under different stocking densities (SD-15 and SD-75) with water being exchanged during the experimental period (Expt. 3).

Comparison of Tilapia Growth under different Ammonia levels

Fig. 7. Comparison of specific growth rates (GR, %/day) with levels of ammonia (mg/L) produced by fish reared under different stocking densities (fish /65L tank) and experimental conditions (with and without the water being exchanged).

Discussion

The results show that increasing stocking density from 1.88 g fish/L to 2.38 g fish/L in both with and without water exchanged groups of fish had no significant effect on growth although in general growth decreased with increasing stocking density. A significantly better growth rate was observed in groups of fish stocked at a density of 2.81 g fish/L when compared to 14.07 g fish/L. The coefficient of variation in size (weight) was significantly influenced by increasing stocking density up to 14.07 g fish/L. Feed conversion ratios were negatively correlated with stocking density. Several studies have found a close relationship between stocking density and growth rates of fish, e.g., negative relationship were observed for the African catfish, Clarias gariepinus (Burchell) fry (Haylor 1991), Nile tilapia, Oreochromis niloticus fry (Huang and Chiu 1997), and
also in the present study. Papst et al. (1992) suggested that in intensive aquaculture the stocking density is an important factor that determines the economic viability of the production system. Furthermore, the size variation of fish may also be affected by stocking density. A linear relationship is usually found between stocking density and growth rate of fish. When the relationship model is valid, it is useful for predicting the optimal stocking density in aquaculture. The effect of stocking density on size variation has been shown to be positive in red tilapia (Watanabe et al. 1990) but negative in Arctic char (Wallace et al. 1988). In the present study, coefficient of variation in size (weight) significantly increased with increasing stocking density. The significant and positive size variations in weight between higher and lower stocking densities in this study support the results obtained by Rubenstein (1981) and Watanabe et al. (1990). The effects of stocking density on size variation also depend on water quality and the biological characteristics of fish (Miao 1992). Also, size variation is related to the social interaction of fish (MacIntosh and De Silva 1984). In crowding conditions, fish that are bigger and maintain a dominant position grow faster. The hypothesis of the present study is that if fish are reared under high density, the threshold for fight is elevated by frequent interactions which lowers discomfort and stress which in turn results in a less persistent dominance hierarchy, more uniform growth and potentially more rapid growth than at low density. However, high stocking density rates may result in high size variation in the present study which is in agreement with the results reported by Huang and Chiu (1997). In the present study FCR was better for lower stocking density indicate less competition and quite life increases aquaculture production whereas at higher density perhaps discomfort, competition, aggression and stress combindly decrease the production efficiency. In addition, tilapia are territorial and aggressive fish (Ali 2001), so density influences growth and size variation which might be by their competition for territories, similar cases were found for African catfish (Haylor 1991).

In the present study, it was found that oxygen concentration of the water did not affect the growth of tilapia although oxygen concentration decreased with increasing stocking density (Fig. 1). In the present study, oxygen concentration of the water was always above 5.0 mg/L and kept similar for all fish at different stocking densities (Figure 4b). Although, in the present study, water exchange did not effect the growth and size variation of O. niloticus between higher (9.38 g fish/L) and lower (1.88 g fish/L) stocking density groups, fish reared at the higher stocking density without water exchange (19 days rearing) changed the coloration of their body (from silver to black). Color change was only found for fish in experiment 1, where stocking density was 9.38 g fish/L (without water exchange), dissolved oxygen was low and ammonia level was high. Why higher stocking density (without water exchange) groups of fish changed coloration of their body is unknown, but it may be that water with higher ammonia concentrations caused by crowdedness and lower dissolved oxygen level could cause this physiological response as a result of environmental perturbation (Wootton 1990).

High density culture of tilapia has been employed to reduce the number of young produced (Allison et al. 1979), increase cannibalism of small fish (Pantastico et al. 1988), and delay age at sexual maturation (Allison et al. 1979). High density also may be useful
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in changing behavior of fish from antagonistic to schooling behavior and this was found to be in the present study where in case of lower stocking density (1.88 g fish/L) fish showed aggressive behavior but in case of higher stocking density (9.38 g fish/L & 14.07 g fish/L) fish there were no such aggressive behavior observed.

In the present study, the levels of ammonia in water increased with increasing stocking density, it was also found that levels of ammonia were significantly higher in fish reared at a higher stocking density without water exchange groups (Figure 6a). Ammonia is the principal nitrogenous compound excreted by aquatic animals and being toxic to fish can limit fish growth (Allan et al. 1990). Excess ammonia can induce detrimental changes in tissue structure, cell function, osmoregulation and reproductive capacity of fish (Harris et al. 1998). In the present study, it was found that increase in stocking density from 15 fish/tank to 75 fish/tank resulted in associated increase in ammonia level (1.48±0.87 mg/L to 26.44±11.4 mg/L) and significantly lower growth rates of *O. niloticus*.

From a management point of view, the price of fish is determined partly by the market demand for supply (size and production) which in turn depends on fish growth and survival rates. The production is the summation of individual weights of all reared fish, or a gross-product of the number of surviving fish and their mean weight (Miao 1992). If the present results would later be used to estimate the production (total biomass in that tank i.e. summation of individual weight of all reared fish) then production should be higher in higher stocking density. Although water exchange did not effect the growth of *O. niloticus*, it has been shown that a higher stocking density without water exchange had a lower growth tendency associated with higher ammonia level (Fig. 7). Comparing the results from the present study under different stocking densities as already mentioned that no significant differences in growth rates were observed fish reared at 10 fish tank⁻¹ compared to 50 fish/tank. However, considering higher production (total biomass will be higher for fish reared in higher density i.e. 50 fish/tank compared to lower density, 10 fish/tank), it may be suggested that when higher stocking density (50 fish/tank) will be maintained then water exchange must be taken in to consideration to reduce the ammonia level and to increase the level of oxygen, so as to avoid any physiological and environmental stress.

References


Hall, London

Arctic charr.

48. x

the pond water.


growth in *Aquaculture and Fisheries Management* 22: 405-422.


Hall, London

Arctic charr.

48. x

the pond water.


growth in *Aquaculture and Fisheries Management* 22: 405-422.


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48. x

the pond water.


growth in *Aquaculture and Fisheries Management* 22: 405-422.


Hall, London

Arctic charr.

48. x

the pond water.


growth in *Aquaculture and Fisheries Management* 22: 405-422.