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Research Article

Effect of stocking density on the physico-chemical characteristics of pond water and survival rate of Nile tilapia (*Oreochromis niloticus)* fish in Bahir Dar, Ethiopia

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Abstract: *The quality of culture water is an important parameter in fish farming as it is the major factor that affects the survival, growth, reproduction and health of the culture animal. With fish stocking density, the physical and chemical characteristics of pond water changes frequently to the extent that affects the performance of the fish. This study aimed to evaluate the effect of stocking density on the physico-chemical characteristics of pond water and the survival rate of Nile tilapia. The study was conducted at the Fishery Research Center in Bahir Dar, Ethiopia, from February to August 2021. Throughout the culture period, the physical water quality parameters of each treatment were recorded twice a day and the measurements of fish growth and chemical parameters of pond water were taken every month. Data recorded during the experimental period was analyzed using Microsoft Excel and the variation between the different means was compared using the SAS software version 9.4. The result of this experiment indicated the existence of significant differences in most water quality parameters at different densities of fish stocking. There was also a difference in the survival of the experimental fishes between treatments. However, the difference in fish survival was not significant and there was no problem on the welfare of fishes. Temperature, dissolved oxygen (DO), pH, conductivity, and salinity ranged between 22.07 °C to 29.43 °C, 3 mg/L to 8.15 mg/L, 6.4 to 10.39, 178 µS/cm to 241µS/cm, and 0.035 psu to 0.12 psu respectively. Secchi depth and chlorophyll-a (Chl-a) ranged between 22 cm to 39 cm and 0.0153µg/L and 0.0513 µg/L respectively. The differences in DO, pH, conductivity, secchi depth, and chl-a between treatments were significant with stocking density ($p < 0.05$). The variation in the level of ammonia was highly significant ($p < 0.001$) between treatments and water quality deteriorated with density. However, the mean ranges of Physico-chemical water quality parameters were at the recommended range for tilapia culture and hence the survival rate of O niloticus at different stocking densities was higher. For the sake of getting higher production per unit area, it is recommended to stock 7 fishes/m^2.*

Keywords: Dissolved oxygen, Pond culture, Stocking density, Survival rate, Water quality
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1. Introduction

The demand for fish has been on the increasing state for the last decades as a result of the over-exploitation of most lakes in Ethiopia. Thus, the current increasing demand for fish in the country can be met through adopting appropriate fish culture practices (Gibtan *et al.*, 2008). Fish farming has great potential in the struggle for improvement of the nutritional status of the population and in the alleviation of poverty of rural people of Ethiopia (Natea *et al.*, 2017). Tilapia has become popular for farmers as it is easy to culture and there is a good demand for fish in the market. Moreover, tilapias (*O. niloticus*) adapt well to the local environment and local feed with higher productivity.

Water is the culture environment for fish where they perform all their bodily functions and they are dependent upon water to breathe, feed and grow, excrete wastes, maintain a salt balance, and reproduce (Bronmark and Hansson, 2005). Water quality is an important factor in aquaculture and can affect growth performance and production. Increased fish density can affect water quality in ponds which also can compromise growth (Herrera, 2015). As density increases, not only growth but also water quality and feed access decrease and limit production performance through its effect on accessing feed and quality of culture water (Schmittou, 2006). Increasing high densities may deteriorate water quality leading to stress (Costas *et al*., 2008; Dagne *et al.*, 2013).

The concentration of dissolved oxygen (DO) was found higher in a lower population, but in the higher population of fish the concentration of DO was found lower (Hasan *et al*., 2010). In fish farming low dissolved oxygen (DO$_{)}$ levels are critical to tilapia and are responsible for more fish kills, either directly or indirectly (Schmittou, 2006). Low concentrations of DO in water can reduce growth, cause stress, increase disease susceptibility, reduce appetite and increase mortality in fish (Bhujel, 2013). The decreased level of DO is associated with increased ammonia, decreased pH, increased nitrate, increased fish metabolism, abundant gill parasites, and numerous other factors, which when combined can significantly reduce fish production performances (Schmittou, 2006). At high density, the concentration of ammonia has increased and that will slow down the growth rate and might increase fish mortality (Francis-Floyd *et al*., 2015). Higher ammonia levels can cause stress and damage to gills and other tissues. On the other hand, fish exposed to low levels of ammonia over time are more susceptible to bacterial infections, have poor growth, and will not tolerate routine handling (El-Sherif *et al*., 2008).

At optimum stocking density, aggressiveness is reduced, and instead, energy can be the channel to growth (Schwedler and Johson, 2000). Under crowded conditions, large fish grow bigger and faster and dominate the population, this is attributed to the cause of social interactions through competition for food and/or space which negatively affects fish growth (Aksungur *et al*., 2007). A considerable advantage of adequate or low stocking density is the significant reduction in mortality, and then high survival (Gomez *et al.*, 2015). Stocking fish beyond an optimum level can also cause a significant increase in fish mortality leading to lower production in pond culture. High density may cause stress in fish, which in turn may suppress immune function in fish and make them more vulnerable to disease. Overpopulation can cause physical damage like fin damage. Studies confirmed that the survival rate was influenced negatively by stocking densities in pond culture (Sophia *et al.*, 2010; Daudpota *et al*., 2014).

Different researchers indicated that the effect of increased stocking density may cause various stress responses, such as increased mortality, increased metabolism, reduced growth rate, reduced feed intake, increased FCR, and poor health in fish (Ellis *et al.*, 2002; Turnbull *et al.*, 2005). Stocking density has affected the health of the fish; by using the lowest initial stocking density, the risk of disease outbreak was also lower (Garcia *et al*., 2013). Stocking density, diet, feeding technique, and management procedures all have strong effects on stress responses, subsequent stress tolerance, health, and the occurrence of aggressive behavior. Strategies to reduce disease susceptibility, minimize stress responses, and avoid aggression are vital (Ashley, 2007). For effective feed, grow, excrete wastes and reproduce in a water body, the aquatic environment has to be optimum and conducive for fishes (Dagne *et al.*, 2013).

Proper stocking density is one of the most important variables in aquaculture to determine the quality of the pond water, the health and survival rate of the fish and the production level. The stocking density of fish in small-scale farmer`s ponds in Ethiopia is 2 fishes/m^2 and the farmers are not producing reasonable amount of fish in their pond. The existing package on the culture of *O. niloticus* in small-scale farmers ponds has not been revised and it is very important to suggest the appropriate stocking density. Information related to the effect of stocking density more than the current trend on the physico-chemical parameter of pond water and the survival and growth of *O. niloticus* in pond culture conditions has not been well studied in Ethiopia. Hence, comparing the different stocking densities more than the current practice and recommending a more suited rate is very important so that farmers can maximize production from their ponds. Hence stocking density is considered as one of the most important variables in aquaculture to determine the quality of the pond water and survival rate of the fish. However, information related to the effect of stocking density on the Physico-chemical parameter of pond water and the survival rate of male *O. niloticus* in pond culture conditions has not been well studied in Ethiopia. Therefore, this study aimed to evaluate the effect of stocking densities on the Physico-chemical parameter of pond water and the survival rate of male *O. niloticus* fingerlings in pond culture.

2. Materials and Methods

2.1. Description of the study area

The pond experiment was conducted at Bahir Dar Fisheries and Other Aquatic Life Research Centre (Ethiopia) located at the southern gulf of Lake Tana with an altitude of 1800m above sea level. According to the Geographic Positioning System (GPS) reading, the study site is located at latitude of 11°36'36" N and longitude of 37°22' 35.5" E.

2.2. Experimental setup and design

The experiment was conducted in concrete ponds with a dimension of 5m by 2m (10 m^2) for a period of six months, from February to August 2021. Each and every experimental pond received water from Lake Tana and collected it in 200 m^3 reservoir. Each pond has a separate water inlet and outlet. The ponds were similar with respect to depth, basin configuration, and pattern including water supply facilities. The ponds have an appropriate inlet overflow and outlet system to maintain a suitable water level. The ponds were first arranged randomly and assigned to each treatment group. The ponds were covered with fine-meshed nylon nets to prevent the entrance of birds and other predators. The experiment was conducted in a semi-intensive pond culture system using male *O. niloticus* fish stocked with three densities (treatments) and each treatment was replicated three times. The treatments were labeled as treatments 1, 2, and 3 for the corresponding stocking density of 3, 5, and 7 fingerlings/m^2, respectively. The total number of experimental fishes (fingerlings) stocked at treatment one (T1) was 30 fingerlings/pond, 50 fingerlings/pond for treatment two (T2), and 70 fingerlings/pond for treatment three (T3).

2.3. Experimental fish selection and measurement

The experimental fish were taken from the hatchery of the research center and only male *O. niloticus* were identified. The sex identification was done manually (hand sexing) through the inspection of their genital organs visually and with the aid of a magnifying hand lens. Fish with visible genital papillae were selected for this experiment. A total of 450 *O. niloticus* fingerings with a mean weight of 26.31 g were collected using seining net. The experimental fishes were fed with commercial feed, from Alema Koudijs PLC, having a crude protein (CP) content of 32%. The fish were fed 5% of their body weight until they attained 100 g and then reduced to 3% as they gained more than 100 g wet weights. Fish growth was recorded every month and the amount of feed provided for the fishes was adjusted accordingly. At the end of the experiment, the fish produced in each pond was totally harvested, individually counted, weighed and lengths were measured.

2.4. Measurement of water quality parameters

As indicated by Tadesse *et al*. (2012) it is important to change one-third of the pond water every month. Accordingly, the water quality was maintained properly through the monthly exchange of water from all the treatment ponds during the experimental period. The temperature, dissolved oxygen (DO), pH, salinity, and conductivity in each pond were measured twice a day using a portable multi-meter Model, HI98194. The transparency of the pond water was measured using a Secchi disk of 20 cm diameter. The daily water quality measurement was taken from the average value of morning (10:00 AM) and afternoon (4:00 PM) records from each treatment pond.

The nutrient analysis of the culture water was done every month using test chemicals and Wagtech photometer 5000. The nutrients were analyzed by taking 0.5 liter of water sample from an experimental pond and filtered it for further analysis. From the filtrate, a 10 ml sub-sample was taken for analysis. To test Ammonia (NH_3), the indophenol method was used, the colorimetric method for total alkalinity and total hardness, and the palintest nitricol method for nitrate (NO_3) and nitrite (NO_2) test was used (APHA, 1999) and the reading was taken by Wagtech photometer 5000.

To identify the plankton compositions, 10 liters of water sample was filtered by 30 µm size plankton net. Before sampling the pond water was mixed properly and the sampled water was taken in the mid-depth, then, the filtered sample was taken and phytoplankton was preserved and fixed with Lugol's solution until the sample took tea color and zooplankton was fixed with formalin solution. Phytoplankton was identified with a compound microscope (Olympus - Japan, S. No.1L0068) using appropriate taxonomic literature on tropical phytoplankton (Bellinger and Sigee, 2010). Zooplankton was identified with a stereomicroscope at a magnification of 45x according to identification keys (Fernando, 2002).

To measure chlorophyll-a (Chl-*a*), 100 ml of water sample was filtered using glass fiber filter papers (GF/F) with 47 mm diameter and 0.7 mm pore size and frozen immediately. Chlorophyll-*a* (Chl-*a*) was extracted in 90% acetone. The sample was centrifuged at 3000 rpm for 10 minutes and the absorbance of the extracted pigment was measured with a DRAWELL UVVIS spectrophotometer at 665 and 750 nm before and after acidification with 0.1 ml of 1 N HCl following a procedure Wetzel and Likens (2000).

The Chl-*a* was calculated according to the equation indicated by Dall'Olmo and Gitelson, (2006).

$$\text{Chl–}a\ (\mu g/L) = \frac{26.73[(665b - 750b) - (665a - 750a)] \times Ve}{Vf \times Z}$$

Where:

- 665b and 750b are absorbance at 665 nm and 750 nm before acidification, respectively
- 665a and 750a are absorbance at 665 and 750 nm after acidification, respectively
- Ve = Volume of extract in ml
- Vf = Volume of sample filtered in a liter
- Z = Path length of the cuvette (1 cm)

2.5. Survival rate and morbidity

The survival rate of the fingerlings was determined after the final harvesting of the fingerlings. The rate of survival and morbidity are computed by using the formula (Biswas *et al*., 2011).

$$Survival\ rate\ (\%) = \frac{Number\ of\ fish\ harvested}{Number\ of\ fish\ stocked} \times 100 \quad [2]$$

$$Morbidity\ (\%) = \frac{Number\ of\ total\ fish\ dead}{Total\ population} \times 100 \quad [3]$$

2.6. Data analysis

The pond water quality as well as fish growth and survival data were recorded and calculations and graphical presentations were made using Microsoft Excel sheet, Office 10. Descriptive analysis was used for the interpretation of all the data gathered in the study using statistical software SAS Students` version 9.4. Means were separated using Least Significant Difference (LSD).

3. Results and Discussion

3.1. Influence of stocking density on water quality parameters

The water quality parameters such as water temperature, dissolved oxygen (DO), pH, conductivity and salinity in all of the treatments at different stocking densities were taken (Table 1). The overall results emanating from this study indicate that the physical parameters of pond water were significantly affected by different stocking densities. As the number of fish increased the quality of the pond water decreased in this experiment. Except for temperature and salinity, the quality of pond water was significantly affected by stocking density ($p < 0.05$).

3.1.1. Dissolved oxygen

The minimum and maximum dissolved oxygen (DO) range was between 3 mg/L to 8.15 mg/L. The DO level was also season dependent where the highest concentration was recorded during the warmer season (April to June) in the area (Fig 1). The level of dissolved oxygen concentration decreased with increasing stocking density. The lowest concentration of dissolved oxygen was observed at the stocking density of 7 fish m^{-2} and the difference was significant ($p < 0.05$) among the treatments. This indicated that the concentration of dissolved oxygen in the pond water was affected by stocking density.

This might be due to the overpopulation of the fish that rapidly consumed dissolved oxygen. High dissolved oxygen is used for the decomposition of organic matter including fish feces, uneaten feed, and high biomass of phytoplankton. This result is in agreement with previous researchers (Garcia *et al*., 2013; Imani and Kasozi, 2014; Herrera, 2015) who found that the dissolved oxygen concentration was lower at the highest stocking density, and using the lowest initial stocking density, the dissolved oxygen level was higher.

Table 1: Water quality parameters as influenced by stocking density

Water quality parameter	Experimental group (stocking density)			P-value
	3 fingerlings/m^2	5 fingerlings/m^2	7 fingerlings /m^2	
Dissolved Oxygen (mg/L)	6.7±0.01^a	5.8±0.18^b	4.98±0.11^c	0.0004
Temperature (^{O}C)	24.96 ± 0.1	25.3 ± 0.2	25.67 ± 0.16	0.0577
pH	8.51± 0.03^a	8.11 ± 0.02^b	7.85 ± 0.13^c	0.0030
Conductivity (µS/cm)	193.18 ± 0.46^c	202.97 ± 0.84^b	210.13 ± 4.06^a	0.0073
Salinity (psu)	0.083 ± 0.02	0.087 ± 0 .001	0.093 ±0. 003	0.0741

Means within a row followed by dissimilar superscript letter (s) are differ significantly at $p < 0.05$.

The availability of a higher concentration of dissolved oxygen was observed at the lowest stocking density. This might contribute to the good growth performance of the fingerlings (182 g). The specific growth rate (SGR) of fish for the highest stocking density was influenced by dissolved oxygen variation and the influence of dissolved oxygen over the growth rate has been well-established for tilapia species (Gullian-Klanian and Arámburu-Adame, 2013). In the highest density, the decreasing growth performance of the fingerlings (153g) might be due to the lower range of dissolved oxygen concentration. This result was also confirmed by Dagne *et al*. (2013) increasing high densities may cause deterioration in water quality leading to stressful conditions.

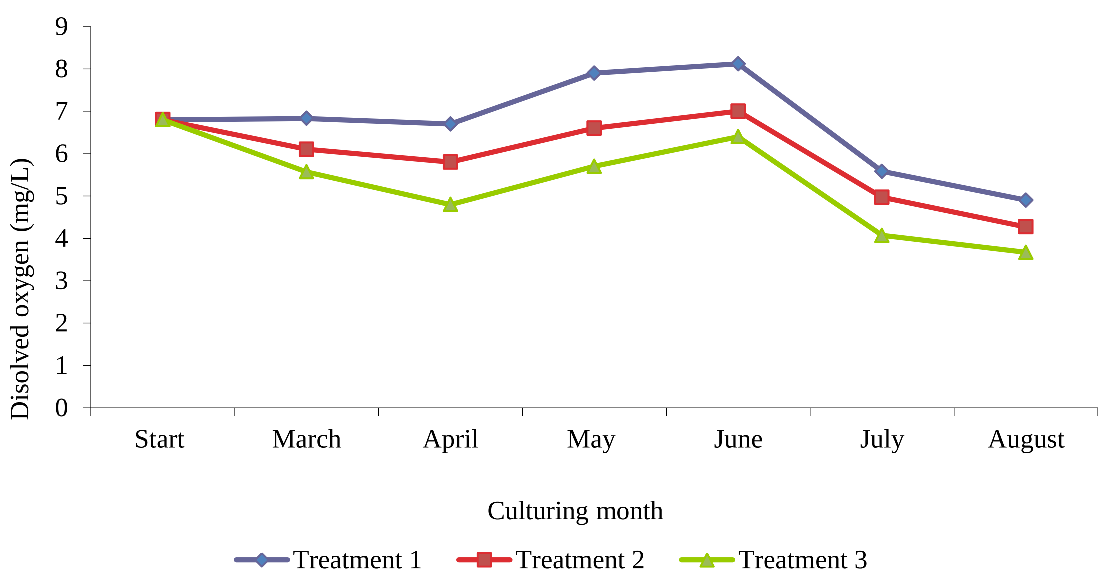

Figure 1: The trend of dissolved oxygen during the culturing months

The trend of dissolved oxygen concentration was included in the month the highest concentration of dissolved oxygen was recorded (8.15 mg/l) in May and Jun and the lowest level of dissolved oxygen concentration was recorded (3 mg/l) in July and August. The cause of lower DO concentration during July and August might be the sunrise (light) was very weak due to this photosynthesis not being properly taking place. During this, time the supply of oxygen decreased, on the contract, the consumption of oxygen increased by fish and microorganisms to the decomposition of organic matter including fish feces and uneaten feed. The present study showed that the concentration of dissolved oxygen in the pond water of mono-sex *O. niloticus* fish was affected by stocking density. However, the mean value of the dissolved oxygen level in 3, 5, and 7 fingerlings/m^2 was 6.7±0.01 mg/L, 5.8±0.18 mg/L, and 4.98±0.11 mg/L respectively not decreasing the standard of dissolved oxygen level for tilapia culture 4-5 mg/l or more (Osofero *et al*., 2009).

3.1.2. Temperature

The average air temperature varied between 22 and 29 ^{0}C and dissolved oxygen was between 3 and 8 mg/l during the experimental period. The surface pond water temperature ranged from 22.07 °C to 29.43 °C. The highest temperature was recorded at the highest stocking density, but the variation was not statistically significant ($p > 0.05$) between the different stocking densities. This result was in line with different scholars (Daudpota *et al*., 2014; Herrera, 2015; Imani and Kasozi, 2014) who reported that the temperature was not affected by different stocking densities.

However, the trend of the temperature was different between months. The highest temperature recorded in May and Jun was 29.43 °C and the lowest at 22.07 °C was recorded in July and August. This result is in agreement with Munni (2013) where the maximum temperature was recorded in June during the assessment of pond water quality for fish culture. During the period of this study, the trend of growth shows that the highest daily growth rate (0.95 g/d) was recorded in May and Jun was might be due to the higher range of temperatures in May and Jun. As temperature increases, the activity of the digestive enzyme increases, which might accelerate the digestion of nutrients, thus resulting in better growth (Mazumder *et al*., 2018). In contrast, the poor daily growth performance (7.6g/d) of the fingerlings, during the period of this study might be due to the lower range of temperatures (22.07 °C) in July and August. During this month uneaten feed particles were observed on the surface of the pond water might result in water pollution, increased biological oxygen demand, and increased bacterial loads. Similar results were reported by Gibtan *et al.* (2008) that the optimum range of temperature for *O. niloticus* is 25 °C to 29°C and a decrease from this level resulted in a reduction in growth. However, the mean water temperature recorded for all treatments (24.96 ± 0.38, 25.63 ± 0.45, and 26.17 ± 0.45 for 3, 5, and 7 fingerlings/m^2) were within the suitable range for tilapia culture (25-32 ºC) and the difference between the stocking densities was not significant ($p > 0.05$).

3.1.3. pH

The maximum and minimum values of pH recorded during the experimental pond were (6.4 to 10.39). The value of pH decreased as stocking density increased and, there was a statistically significant difference ($p < 0.05$) between stocking density. Because the same surface area increased the number of fish, the concentration of ammonia also increased might be the reason for the higher stocking density reduced pH, and increased acidity level in the pond. This is in agreement with (Imani and Kasozi, 2014) reported pH values were lower at the highest density of 6.5±0.3 than pH for the lowest density7.4±0.2. Herrera (2015) also reported the pH was increased (7.38) at the lowest density than the pH at the highest density (6.8). In addition, an increase in stocking density resulted in a reduction in dissolved oxygen and pH this is in line with Manduca *et al*. (2021). However, the mean pH for 3, 5, and 7 fingerlings m^{-2} (8.51± 0.03, 8.11 ± 0.02, and 7.85 ± 0.13), respectively were within the permissible optimum range (6.5 to 11.0) for tilapia culture.

3.1.4. Electrical conductivity

The electrical conductivity (µS/cm) value recorded in the three treatments varied from178 µS/cm to 241µS/cm and the variation between treatments was a significant difference ($p < 0.05$) between treatments. In the present study in the lower stocking density (3 Fingerlings m^{-2}) the concentration of dissolved oxygen increased the growth of fish also increased in the contract in the higher stocking density (7 fingerlings m^{-2}) ammonia concentration and electrical conductivity increased, the growth of fish decreased. This is in line with (Makori *et al.*, 2017) as DO increased, the growth rate of tilapia increased. But, an increase in conductivity, pH, and ammonia decreased fish growth rate. However, the mean electrical conductivity for 3, 5, and 7 fingerlings/m^2 (193.18 ± 0.46 µS/cm, 202.97 ± 0.84 µS/cm, and 210.13 ± 4.1 µS/cm) was found within the acceptable range. The mean water conductivity would be between 150 and 500 µS cm-1 and safe for fish culture (Russell *et al*., 2011).

3.1.5. Salt concentration (salinity)

The pond water salinity level ranged from 0.035 psu to 0.12 psu (Table 1). There was slight variation between treatments; the highest salinity was recorded on treatment with the highest stocking density. It might be in the highest stocking density the level of acidity and ammonia concentration were increased relative to lower stocking density and the reason for to increase in salt concentration in the highest stocking density. But the mean salt concentration of the pond water during the experimental period was 0.083 ± 0.02 psu, 0.087 ± .0043 psu, and 0.093 ± 003 psu for ponds with 3, 5, and 7 fishes/m^2, respectively. However the difference between the treatments was not statistically significant ($p > 0.05$).

The Secchi depth (cm) reading ranged from 22 cm to 39 cm. The Secchi depth reading had shown direct relationships with stocking density (Table 2) and the difference in Secchi reading between treatments was statistically significant ($p < 0.05$). In the present investigation, the transparency of the pond water was increased with increasing stocking density, and the level of Secchi depth was positively related to stocking density. The highest value of Secchi depth level (39 cm) was recorded in the highest stocking density (7 fish/m^2), while the lowest Secchi depth level (22 cm) was recorded in the lowest stocking density (3 fish/m2) this might be the concentration of phytoplankton measured a chlorophyll-a (Chl-*a*) showed a high level and the transparency of pond water decreased in the lower stocking density (3 fish/m^2), while a low level of chlorophyll-*a* was recorded in the higher stocking density (7fish/m^2) and also, the water transparency increased. It might be the algal biomass was eaten by fish in the high stocking density. A similar result was reported by Kubitza (2000) and indicated that the lower the stocking density, the more the contribution of planktons. At higher stocking density the pond water was more transparent (Bellinger and Sigee, 2010; Hasan *et al.*, 2010; Sophia *et al.*, 2010).

The maximum and minimum concentration of phytoplankton measured as Chl-a (µg/L) was 0.053µg/L and 0.013 µg/L and the mean value of Chl-*a* was 0.047±0.002µg/L, 0.035±0.003µg/L, and 0.02±0.03µg/L in treatments one, two and three, respectively and the difference was statistically significant ($p < 0.05$) between lower stocking density (3 fish/m^2) and higher ones (5 and 7 fishes/m^2). The highest level of chlorophyll-a (Chl-*a*) shown in the lower stocking might contribute to higher growth performance fingerlings. The trend of Secchi depth level within a six-month culturing period the lowest Secchi depth level was recorded in May and Jun relatively another month. The algal production and suspended organic matter might be increased in the fish pond water during these two months.

Table 2: Secchi depth and biomass of chlorophyll-*a* at different stocking densities of the *O. niloticus* fingerlings

Water quality parameter	Experimental group/ stocking densities		
	3 Fingerlings/m^2	5 Fingerlings/m^2	7 Fingerlings/m^2
Secchi depth (cm)	28.5 ± 0.09^c	30.5±0.09^b	32.44±0.33^a
Chlorophyll-*a* (µg/L)	0.047±0.002^a	0.035±0.003^b	0.02±0.03^c

Means within a row followed by dissimilar letter (s) are significantly different at $p < 0.05$.

3.2. Influence of stocking density on pond water nutrients

3.2.1. Ammonia

The concentration of ammonia (mg/L) recorded at three treatments varied between 0.01 mg/L to 0.31 mg/L. The concentration of ammonia during the study period was increased with increasing stocking density (Table 3). The difference in ammonia concentrations between the different stocking densities was significant ($p < 0.05$). The highest concentration of ammonia was manifested in the highest stocking density of 7 fish/m^2 (Figure 2). This might be due to the increased biomass that resulted in the release of high waste products into the environment. This result is in line with Francis-Floyd *et al*. (2015) who reported the concentration of ammonia increased in fish stoked at high density. Gullian-Klanian and Arámburu-Adame (2013) reported that the SGR of the highest stocking density was influenced by ammonia concentration (0.25 mg/l) than the lowest stocking density (0.15 mg/l) and ammonia concentration was affected by different stocking density. In the present study mean daily growth rate and specific growth rate of *O. niloticus* fingerling decreased as the ammonia concentration of the pond water increased. The one-factor retarded fish growth at the highest stocking density was ammonia concentration. The results of El-Sherif and El-Feky (2008) also agree with the findings of the present study and indicate that at a high concentration of ammonia, the growth rates of the fish slow down and mortality is also increased.

During the experimental period in the highest stocking density, the concentration of dissolved oxygen and the level of pH decreased. This causes the acidity level of the pond water to increase, hence causing the level of ammonia concentration to rise. These results were in agreement with Schmittou (2006) who reported that ammonia toxicity in tilapia culture is closely correlated with pH and to a lesser extent, water temperature, and dissolved oxygen concentration. Increased stocking density may have caused stress in the fish which in turn may have reduced the growth at the highest density. Reduced water quality may likely have contributed to the stress Herrera (2015).

The trends of concentration of ammonia increased monthly which might be due to increasing the uneaten food particles, fecal wastes of fish, and plankton. Fish is a poikilothermic animal the temperature difference affects the physiology of the fish. Because different physico-chemical parameters of water like temperature, dissolved oxygen, free CO_2, transparency, and pH are generally considered to have primary importance in fish culture (Islam *et al*., 2006). Dagne *et al*. (2013) also indicated that the performance of fish in aquaculture is extremely dependent on the water quality.

3.2.2. Total alkalinity and hardness of pond water

The minimum and the maximum pond water total alkalinity and hardness levels ranged from 79.75 to 81.87 and 67.12 to 73 mg/L$CaCO_3$, respectively (Table 3). As a general rule, the most productive waters for fish culture have a hardness and alkalinity of approximately the same magnitude, because calcium, magnesium, bicarbonate, and carbonate ions in water are derived in equivalent quantities from the solution of limestone in geological deposits. However, in some waters alkalinity may exceed its hardness and vice versa (Boyd *et al.,* 2016). In the present study, there are slight variations between treatment, total alkalinity, and total hardness in the pond water decrease with increasing stocking density. In the higher stocking density the level of pH or acidity and the concentration of ammonia also increase which might be the reason decrease in the level of alkalinity and hardness in the higher stocking density and the contrast in the lower stocking density due to decreasing acidity level of pond water, increase the level of total alkalinity and hardness. However, the mean pond water total alkalinity and hardness level record was not a statistically significant difference between treatments in the present study ($p > 0.05$). Because, total alkalinity and hardness are not greatly affected by biological activity or aqua-cultural operations, and the initial concentrations in ponds are determined by their level in the water supply; any changes are largely the result of rainfall and evaporation (Conte, 2004). Total Alkalinity and hardness recorded in the experimental pond were in the suitable range for tilapia culture. The desired total alkalinity level for most aquaculture species lies between 50-150 mg/L CaCO3 and suitable hardness for soft water ranges between 20 to 75mg/l (James, 2000).

Table 3: Nutrient level of pond water at different stocking densities

Water quality parameter	Experimental group/ stocking densities			P-value
	3 Fishes/m^2	5 Fishes/m^2	7 Fishes/m^2	
Ammonia (mg/L)	0.11±0.002^c	0.15±0.003^b	0.19±0.005^a	<0.0001
Total Alkalinity (mg/L)	81.35 ±0.29	81.08 ±0.55	79.66±0.36	0.0592
Total Hardness (mg/L)	70.67±1.5	68.54±0.7	67.37±0.19	0.1334
Nitrate (mg/L)	15.91±1.1	20.62±1.9	22.83±4.4	0.2949
Nitrite (mg/L)	0.0008±0.0004	0.0011±0.00005	0.0024±0.0009	0.2664

Means within a row followed by dissimilar letter (s) are significantly different at $p < 0.05$

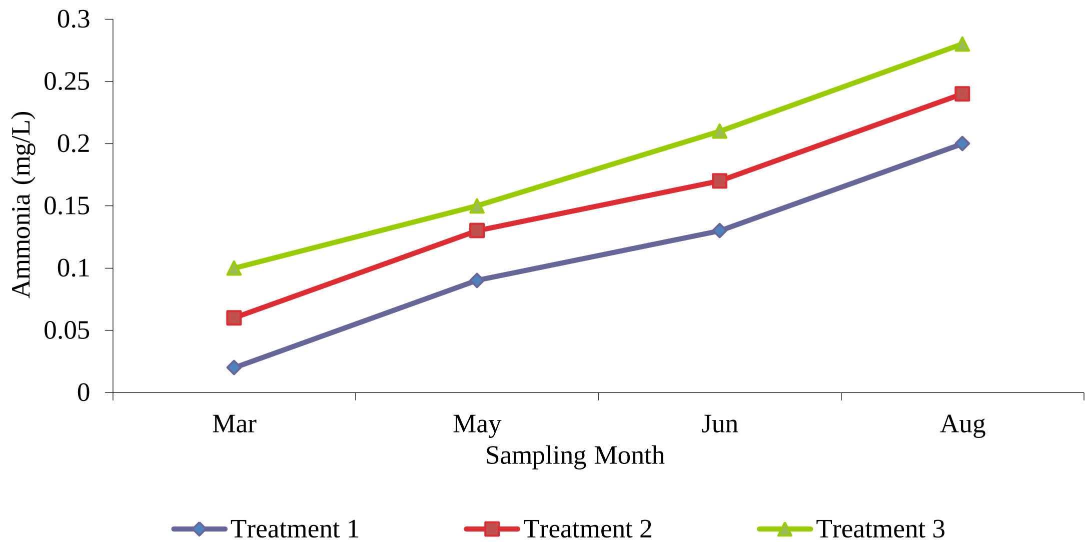

Figure 1. Temporal variation of ammonia concentration

3.2.3. Nitrate (NO_3) and Nitrite (NO_2)

The minimum and the maximum pond water nitrate and nitrite ranged from 13.75 to 31.25 mg/L and from 0 up to 0.0014 mg/L, respectively (Table 3). Although, the difference is not significant' there exists slight variation in values of nitrite and nitrate between the stocking densities. As the stocking density increased, the Nitrate and Nitrite levels also increased; because, Nitrite is converted to nitrate (NO_3) by nitrifying bacteria, and a nitrite can become a nitrate by the process of oxidation, and similarly, a nitrate can become a nitrite by the process of reduction (Crab *et al*., 2007). The reason is that an increase in nitrate and nitrite in the pond water with increased stocking density might increase the concentration of ammonia, in the higher stocking density. However, the mean pond water nitrate and nitrite were within the suitable range for tilapia culture. Nitrate levels from 0 – 40 ppm are generally safe for fish.

3.3. Mortality and survival rate of *O. niloticus* fingerlings at different stocking densities

During the six-month experimental period, mortality was recorded in all the treatments with varying stocking densities. The highest morbidity (9.05%) was observed on fishes with highest stocking density (7 fishes/m^2) and the lowest morbidity (6.66%) was observed on treatment 1 (5 fishes/m^2). The rate of morbidity in all treatments with different stocking densities showed temporal variation between sampling months (Table 4). The highest overall record of morbidity was recorded during August with a morbidity level of 3.04 % followed by July that was 2.73% (Table 4). The morbidity percentage was 6.67%, 7.33% and 9.05% for treatments 1, 2 and 3, respectively. The result indicated that highest rate of stocking result in higher morbidity. The overall morbidity of the experimental fish was only 8%, which is very low compared to other similar studies (Imani and Kasozi, 2014; Herrera, 2015).

The highest mortality during August and July might be due to the decrease in dissolved oxygen level and the associated with the increase in the concentration of ammonia in the water during these months compared to the other months (Fig 2).

The survival rate of the fingerlings varied between 88% and 98%. This might be due to the high competition for food and space among the fish, the confinement, and the consequence of behavioural changes. However, the difference was not significant ($p > 0.05$) and stocking density did not affect the survival rate of the fingerlings. The same situation was reported in other studies (Gullian-Klanian and Arámburu-Adame, 2013; Imani and Kasozi, 2014; Herrera, 2015). The overall highest survival rate recorded in the present study may be attributed to the proper handling of fish and possibly good water quality. This finding indicates that stocking density has also a limited effect on fish survival.

Table 4: Mortality recorded at different stocking densities and months

Treatment	Dead fish							Survival rate (%)
	March	April	May	Jun	July	August	Morbidity (%)	
3 Fingerlings/m^2	0	0	0	1	3	2	6.67	93.1%
5 Fingerlings/m^2	1	1	1	1	3	4	7.33^{b}	92.67%
7 Fingerlings/m^2	2	4	0	0	6	7	9.05^{a}	90.95%
Monthly total (No.)	3	5	1	2	12	13		
Morbidity (%)	1.33	1.12	0.23	0.45	2.73	3.04	8	

Means within a coloumn followed by dissimilar letter (s) are significantly different at $p < 0.05$

3.4. Fish growth as influenced by stocking density

The experimental fish in all the treatments showed an increment both in length and weight, with a daily growth rate of 0.9, 0.79 and 0.93g/day for treatments 1, 2 and 3, respectively (Table 5). There exists a difference in growth among the treatment groups. The daily growth rate (DGR) and percentage weight gain (PWG) were also varied between treatments. The specific growth rate (SGR) was 1.11 for treatment 1 and 1.05 and 1.01 for treatments 2 and 3, respectively (Table 5). The mean values of all the growth parameters (DGR, PWG and SGR) of the experimental fishes assigned to different treatment groups showed highly significant difference ($p<0.001$).

The lower growth performance of *O. niloticus* observed at higher stocking in this experiment might be due to the high level of energy expended in search of food and stress caused by higher competition for food, space and other resources. Ashagrie *et al*. (2008) also reported that high stocking density impairs the growth of *O. niloticus* fishes.

Table 5: Growth performance of experimental fishes with stocking density

Growth parameters	3 Fishes/m^2	5 Fishes/m^2	7 Fishes/m^2	P -value
Initial fish length (cm)	11.42 ± 0.12	11.44 ± 0.04	11.47 ± 0.06	0.97
Final fish length (cm)	23.46 ± 0.03^{a}	21.56 ± 0.21^{b}	20.23 ± 0.07^{c}	< 0.0001
Initial fish weight (g)	26.31 ± 0.06	26.28 ± 0.08	26.33 ± 0.1	0.89
Final fish weight (g)	182.58 ± 0.39^{a}	163.33 ± 0.78^{b}	153.57 ± 0.34^{c}	< 0.0001
Percentage weight gain (%)	593.95 ± 2.3^{a}	521.53 ± 4.4^{b}	483.19 ± 1.8^{c}	0.001
Daily growth rate (g/day)	0.9 ± 0.01^{a}	0.79 ± 0.005^{b}	0.73 ± 0.001^{c}	< 0.0001
Specific growth rate	1.11 ± 0.002^{a}	1.05 ± 0.004^{b}	1.01 ± 0.00^{c}	< 0.001

Means within a row followed by dissimilar superscript letter(s) differs significantly at $p < 0.01$

4. Conclusion and Recommendations

Different stocking densities influenced significantly the secchi depth, dissolved oxygen, pH, conductivity and the ammonia level of pond water. Temperature, salinity, total alkalinity, total hardness, nitrite and nitrate concentration did not show significant variation between treatments. At higher stocking density, the transparency of pond water increased, and the concentration of phytoplankton measured as chlorophyll-*a* (Chl-*a*) gets lowered. Thus, the level of dissolved oxygen dropped. This might happen due to the higher fish population that grazes the algal biomass and prohibits the algae from multiplying effectively. The overall results emanating from this study indicated that good pond water quality was observed at lower stocking densities. The quality of pond water was deteriorating on treatments with higher stocking density. This might be due to the higher fish numbers that contribute to the excretion of metabolites to the pond water. As the number of fish increases, the plankton will not get a chance to grow and could not consume the nutrient in the pond. However, the nutrient load was not beyond the tolerable range for tilapia and hence the survival rate was good. Therefore further investigation is required to limit the appropriate stocking density to culture fish in similar ponds and management practices without compromising fish production and welfare.

Data availability statement

Data will be made available on request.

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Conflicts of interest

The authors declared that there is no conflict of interest.

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