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Preliminary Study on the Effects of High Dissolved Oxygen on Growth, Survival, and Feeding Rate of *Oreochromis niloticus* (Cichliformes: Oreochromis) using *Egeria densa* as Natural Oxygenator

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ABSTRACT

A major protein source in Malaysia is *Oreochromis niloticus*, and its development is greatly influenced by the level of dissolved oxygen (DO). This research was done to determine the effects of DO on *O. niloticus* development, survival and feeding rate, as well as the potential of *Egeria densa* as a natural oxygenator in place of oxygen pumps. This study hypothesises that *E. densa* serves as a natural oxygenator, enhancing DO levels and promoting growth, survival, and feeding rate in *O. niloticus*. Three tanks with 50 *O. niloticus* each were assigned as Tank A (control), Tank B (*E. densa*), and Tank C (with an oxygen pump). The fish were fed bran twice daily, with feed amounts adjusted weekly based on mean body weight, over a 12-week experimental period. Survival rate (SR%), weight-gained (WG%), specific growth-rates (SGR%), feed conversion ratio (FCR%) and mortality-rate (MR%) were manually calculated, while the feed utilisation, growth performance, and water parameters were statistically analysed using Minitab 21 and Microsoft Excel. Overall, mean length and weight differed significantly ($p<0.05$) among tanks ($p=0.000$).

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In Tank C, length differed significantly between fish with and without oxygen (p=0.006), whereas weight did not (p=0.442; p>0.05). The length-weight regression analysis (LWRs) showed DO has a significant impact on *O. niloticus*' growth rate with negative allometric (Tank A, b=2.6828; Tank B, b=2.5248), and isometric (Tank C, b=3.0084). The highest SR% with the lowest MR% and FCR% was shown in Tank C. *E. densa* respiration competes for DO, making it unsuitable to replace air pumps in aquaculture systems.

Keywords: Dissolved oxygen, *Egeria densa*, growth rate, hyperoxia, *Oreochromis niloticus*

INTRODUCTION

Freshwater aquaculture involves rearing aquatic species in inland waters such as ponds, lakes, rivers, and reservoirs, including brackish water for economic purposes (Li & Liu, 2019). Rising demand for aquaculture protein drives expansion into coastal and offshore areas, enhancing food security and reducing pressure on wild stocks (Gimpel et al., 2018). Fish farming supports economic growth by providing high-quality protein and is often subsidised due to its profitability (Sangirova et al., 2020). According to Kuljanishvili et al. (2021), due to its economic worth and effective production, *Oreochromis niloticus*, or commonly known as Nile tilapia, is the most cultured tilapia species. In Malaysia, this species accounts for 90% production of tilapia as a reasonable protein source (Saba et al., 2020). Its acceptance comes from exponential growth (harvest size in six months), adjustability to changed agricultural practices, high stocking tolerance, and minor protein diet needs, contrasted to marine fish (Mohamad et al., 2021).

According to Nariswari et al. (2020), the growth of *O. niloticus* is affected by stocking density, the rate of feeding, frequency and water quality (DO, temperature, pH and total dissolved solids (TDS)). Lower stocking densities normally improve growth, survival and productivity, making optimal density necessary for maximising production and profits (Abaho et al., 2020). Feed type, ration size and frequency also affect growth and feed conversion ratio of the fish (Mabroke et al., 2021). Knowing feeding patterns advances the fish management, diet formulation and sustainable aquaculture (Tesfahun & Temesgen, 2018). Appropriate feed management ensures economically efficient and eco-friendly production with studies of optimising feeding frequency in clear water systems (Mabroke et al., 2021).

The quality of water is essential for aquaculture, directly influencing fish condition and growth (Mannan et al., 2012). Refer to Ercan et al. (2015) and Sriyasak et al. (2015), the DO, pH, salinity, temperature and TDS are the factors that influence consuming, growth and development, disease tolerance and survival rates of the Nile tilapia. This species thrives at 28 °C-32 °C with a pH of 7-9 and DO above 3 mg L^{-1} (Nehemia, 2012). Excess phosphorus and nitrogen promote algal blooms, which increase turbidity, reduce light penetration, limit photosynthesis and gas exchange, and ultimately deplete dissolved oxygen (DO) (Schmidt et al., 2019). Fish survival requires sufficient DO, with 400 ppm as the upper exposure limit for freshwater fish (Munni et al., 2013).

Egeria densa is a freshwater plant native to temperate and subtropical regions of South America. It is unique due to its paired scale leaves, translucent idioblasts, and fiberless leaf margins (Shamso & Hosni, 2020). Currently, it is found worldwide except in Antarctica (Drexler et al., 2021), which can grow up to 5 meters long in water up to 4 meters deep. It has trailing stems that produce roots regularly with oval to oblong leaves, 1.5-4 cm long, that grow in clusters of 4-5 along the stems. Lower leaves appear in pairs or groups of three, while upper leaves form clusters of 4-8, creating dense tips that reach the water's surface (Yarrow et al., 2009). It thrives in lakes, ponds, and slow-moving waters, usually at depths of 0.1-1.5 meters (Białowiec et al., 2019). *E. densa* was introduced as an oxygenator before becoming an invasive species (Shamso & Hosni, 2020). In order to survive in low CO_2 environments, it uses Phosphoenolpyruvate carboxylase (PEPC) to efficiently take up bicarbonate (HCO_3) (Lara et al., 2002).

To increase oxygen levels and improve water quality, aeration of freshwater is vital (Febiyanto, 2020). Circulating water and dissolved oxygen levels must be maintained during live fish transport, whereas in standing water, aeration aids in oxygen balance (Rifat et al., 2019; Yao et al., 2022). In fish farming, the aeration systems include paddle wheels and spiral aerators (Tanveer et al., 2018). Expanding the water surface enhances oxygen intake, while water mixing ensures even distribution and prevents over-oxygenation. Mixing during the day helps conserve oxygen for nighttime use (Rifat et al., 2019).

This study aims to demonstrate the importance of oxygen levels in fish growth, survival, and feeding rates, specifically in *Oreochromis niloticus*. The findings may indirectly impact farming and fishing income. Hence, the objectives of this study are to assess the effect of high oxygen levels on the growth, survival, and feeding rate of *Oreochromis niloticus* and to evaluate the effectiveness of *Egeria densa* as a natural oxygenator in replacing oxygen pumps in aquaculture.

MATERIALS AND METHODS

Experimental Designs and Sample Identification

The experiment was conducted at Kolej Seri Menanti 6, Kuala Pilah campus, UiTM Negeri Sembilan from November 11, 2021, to January 19, 2022. Three 500-gallon circular tanks (60-inch top diameter, 52-inch bottom diameter, 51-inch height) were filled with 2000 litres of tap water using 120 ml of anti-chlorine. The tanks labelled A, B, and C were placed outdoors under sunlight and covered with a black net for fish protection.

A total of 150 *Oreochromis niloticus* fry were purchased from a supplier in Kg Beting, Ulu Muar district. Their identification followed Tilapia and Trout (Alam et al., 2017) and

Zhu et al. (2016), using caudal fin banding and body colour patterns. Nile tilapia have distinct vertical bands on the caudal fin and a pink or grey throat (Alam et al., 2017). Each tank received 50 fry for observation. Their initial size ranged from 3.02-3.67 cm in standard length (SL), 1.4-1.6 cm in body depth (BD), and 1.4-2.4 g in weight (W). Table 1 provides characteristics of each experimental tank as refer Figure 1, Figure 2 and Figure 3 in this study. In Tank B, 150 bunches (200 grams) of *E. densa* were added, and the same amount was used throughout the experiment to ensure consistency.

Ten *O. niloticus* were randomly selected from each tank and measured twice weekly for SL, BD, and weight using a ruler and a digital scale for 12 weeks. A pre-weighed plastic beaker containing 500 mL of water was used to weigh fish, thereby minimising handling stress. The morphometric measurements were illustrated in Figure 4. The snout tip to the caudal fin base measurement was taken for SL measurement, while the maximum vertical distance between the dorsal and ventral margins was measured for BD measurement (Jayraj et al., 2019).

In order to record mortality rates and fish survival, the tanks were monitored daily. To ensure consistent water quality across the treatments, the DO Meter (JPB 70 A) and pH/TDS meter were used to record temperature, DO, TDS, and pH. Readings were taken thrice weekly in the morning, afternoon, and evening (weeks 1 to 12). From weeks 10-12, Tank C's oxygen supply was cut off, and observations continued.

Table 1
Characteristics of each experimental tank in the study (11 November 2021 - 19 January 2022)

Tank	Components
Tank A	Treated water + *O. niloticus*
Tank B	Treated water + *Egeria densa* (150 bunches) + *O. niloticus*
Tank C	Treated water + oxygen pump and filter + *O. niloticus*

Figure 1. Tank A (control) containing treated water and *O. niloticus*

Figure 2. Tank B containing treated water, *E. densa* (150 bunches) and *O. niloticus*

Figure 3. Tank C equipped with an oxygen pump and filter, containing treated water and *O. niloticus*

Feeding Management

O. niloticus was fed bran twice daily at 0900 and 1700. Fry received crumble feed, while fingerlings were given floating pellets. Different feed sizes were used to suit the mouth size and feeding habits of fry and fingerlings, and floating bran is easy to collect for weighing, allowing fair comparison of feed conversion. The feed amount was calculated using the feed ration formula and adjusted weekly based on average fish weight. The feeding rate (%) followed the FAO (2021) schedule (Table 2). Leftover feed was removed after an hour, dried, and used to calculate the feed conversion ratio (FCR) using Equation 1 for each tank.

$$\text{Feed ration} = \text{average fish size (weight)} \times \text{feed rate (\%)} \times \text{total number of fish} \quad [1]$$

Table 2
Feeding rate (%) of Oreochromis niloticus *during fry, fingerling, juvenile and grower stages*

Life Stage	Fish Size (g)	Feeding Rate (% body weight)
Fry	1 - 5	10 - 6
Fingerling	5 - 20	6 - 4
Juvenile	20 - 100	4 - 3
Grower	100 - 250	3 - 2
	> 250	2 - 1.5

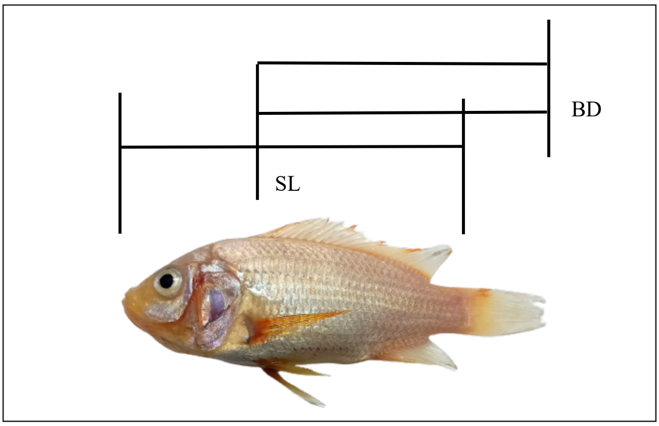

Figure 4. Measurement of standard SL, BD, and W of *O. niloticus*

Data Analysis

The survival rate (SR%), weight gained (WG%), specific growth rates (SGR%), feed conversion ratio (FCR%), and mortality rate (MR%) can be expressed by the Equations 2, 3, 4, 5, and 6. These were used to assess growth performance and feed utilisation.

$$\text{SR\%} = (\text{Number of fish at the end of the experiment}/\text{Number of fish at the beginning of the experiment}) \times 100 \quad [2]$$

$$\text{WG\%} = [(\text{Final weight, g}—\text{Initial weight, g})/\text{Initial weight, g}] \times 100 \quad [3]$$

$$\text{SGR\%} = [(\text{Final weight, g}—\text{Initial weight, g})/\text{No. of day}] \times 100 \quad [4]$$

$$\text{FCR\%} = [\text{Feed fed (g)}/\text{Weight gained (g)}] \times 100 \quad [5]$$

$$MR\% = [\text{Number of dead fish/ Total number of fish at the beginning of the experiment}] \times 100 \qquad [6]$$

Fish length and weight recorded after 11 weeks were tabulated. The length-weight relationship (L, cm) and weight (W, g) were calculated using the power regression equation $W = a L^b$, where a is the intercept and b is the regression coefficient. The relationship coefficient (r^2) determined the association between length and weight. Growth was assessed based on b: isometric growth (b=3), positive allometric growth (b>3), and negative allometric growth (b<3) (Morey et al., 2003).

2-sample-t-test and One-way ANOVA were used to analyse the effect of DO on fish growth and feed efficiency in *O. niloticus*. This analysis was performed using the Minitab 21 software and was considered at a significance level of 5% (P<0.05). Microsoft Excel was used to save the data and create graphs.

RESULTS

Dissolved Oxygen Measurements

Tank C had the highest DO level (12.32 ± 3.83^c mg/L), followed by Tank B (10.84 ± 3.06^b mg/L) and Tank A (8.82 ± 2.23^a mg/L), with a 3.52 mg/L difference between the highest and lowest levels. The analysis shows significant variation in DO levels across tanks, but the data's accuracy is limited as it was collected only during the daytime.

Mean Length and Weight of Treated and Untreated *Oreochromis niloticus*

Figures 5 and 6 compare the mean length (cm) and weight (g) of *O. niloticus* in Tank A (control), Tank B, and Tank C. The mean length of fish in Tank A (6.157 cm) and Tank B (5.7463 cm) showed no significant difference (p=0.055; p>0.05). However, Tank C (7.089 cm) had a significantly higher mean length (p=0.00; p<0.05) compared to both Tank A and Tank B. Similarly, fish weight in Tank A (9.095 g) and Tank B (7.08 g) showed no significant difference (p=0.05; p>0.05), but Tank C (15.701 g) had a significantly higher weight (p=0.00; p<0.05). Overall, there were significant differences in the mean length (p=0.000, p<0.05) and mean weight (p=0.000, p<0.05) of the fish.

To highlight the importance of DO, the oxygen supply in Tank C was cut off for three weeks. The mean length and weight of *O. niloticus* were then compared to the same samples when oxygen was supplied in the previous three weeks. Figures 7 and 8 show the mean length and weight ranges in Tank C with oxygen (TCO) and without oxygen (TCWO). The mean length and weight range in TCO (0.71 cm; 4.65 g) were higher than in TCWO (0.33 cm; 3.02 g).

There was a significant difference in mean length ($p=0.006$, $p<0.05$), but no significant difference in mean weight ($p=0.442$, $p>0.05$). Despite the lack of a significant difference, a decrease in weight was observed, indicating a possible effect of dissolved oxygen.

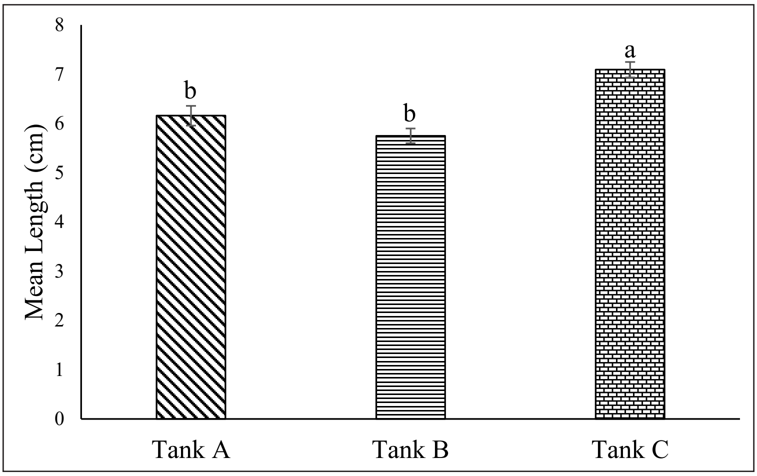

Figure 5. Comparisons between Tank A, Tank B and Tank C for the mean length (cm) of *Oreochromis niloticus*
Note. Tank A = Control; Tank B = Treatment 1 (*Egeria densa*); Tank C = Treatment 2 (supplied with oxygen); Means (± SE) marked with different letters "a" and "b" are significantly different at $p=0.05$ (Tukey's test), confidence level=95%

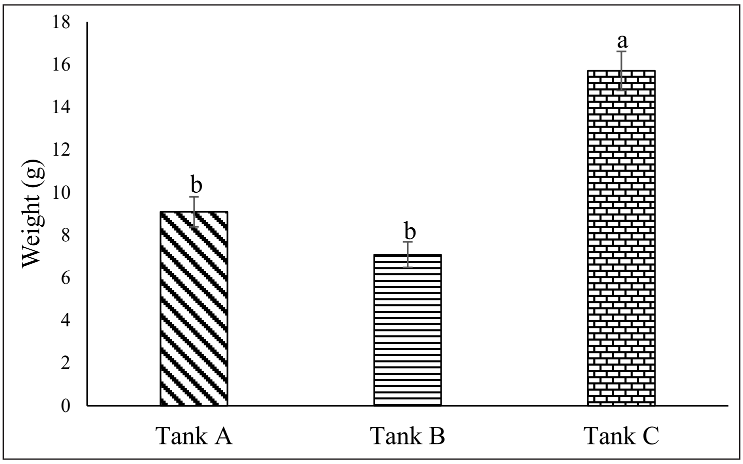

Figure 6. Comparisons between Tank A, Tank B and Tank C for the weight (g) of *Oreochromis niloticus*
Note. Tank A = Control; Tank B = Treatment 1 (*Egeria densa*); Tank C = Treatment 2 (supplied with oxygen); Means (± SE) marked with different letters "a" and "b" are significantly different at $p=0.05$ (Tukey's test), confidence level = 95%

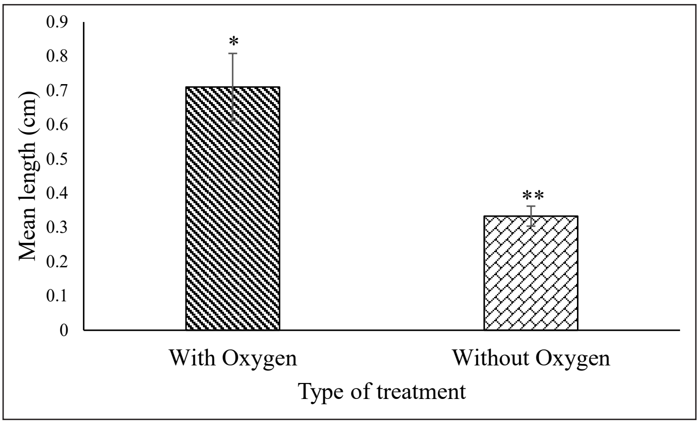

Figure 7. Mean length of *Oreochromis niloticus* in Tank C with and without oxygen
Note. Bars represent mean ± SE. Asterisk (*) indicates a statistically significant difference between groups at $p<0.05$ (Tukey's test). cm = in centimetres

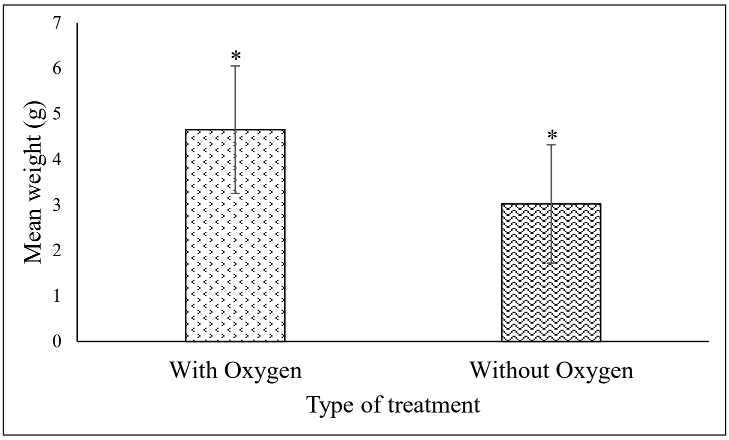

Figure 8. Mean weight of *Oreochromis niloticus* in Tank C with and without oxygen
Note. Bars represent mean ± SE. Asterisk (*) indicates a statistically significant difference between groups at $p<0.05$ (Tukey's test). g = in grams

Length-weight Regression Analysis of *Oreochromis niloticus*

Figures 9, 10, and 11 show the length-weight (LW) regression analysis of *O. niloticus* in Tanks A, B, and C. The fish in Tanks A and B exhibited negative allometric growth, with b values 2.6828 and 2.5248 ($b<3$), respectively. Oppositely, Tank C showed isometric growth, with $b=3.0084$ ($b=3$). The LW relationship equations were $W=0.061 \times L^{2.6828}$ ($R^2=0.9175$) for Tank A, $W=0.0779 \times L^{2.5248}$ ($R^2=0.9177$) for Tank B, and $W=0.0348 \times L^{3.0084}$ ($R^2=0.962$) for Tank C. These equations help determine the relationship between fish length and weight.

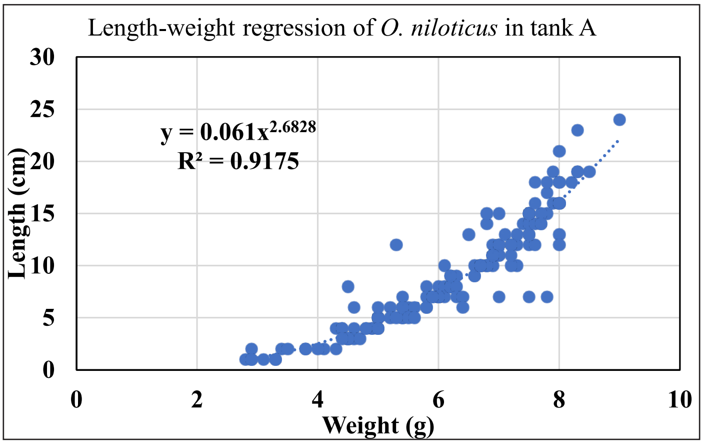

Figure 9. Length-weight regression of *Oreochromis niloticus* in Tank A (control) shows negative allometric growth (b<3)

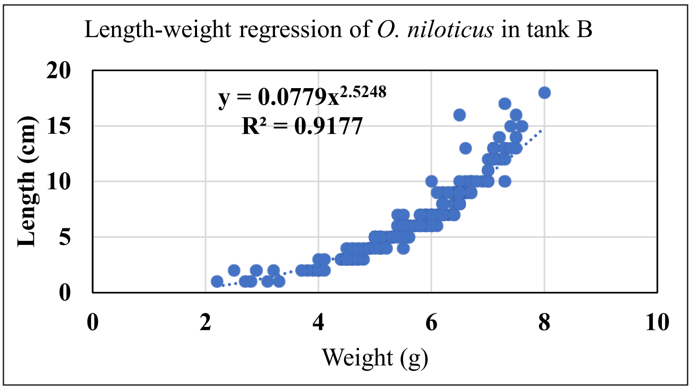

Figure 10. Length-weight regression of *Oreochromis niloticus* in Tank B (*Egeria densa*) shows negative allometric growth (b<3)

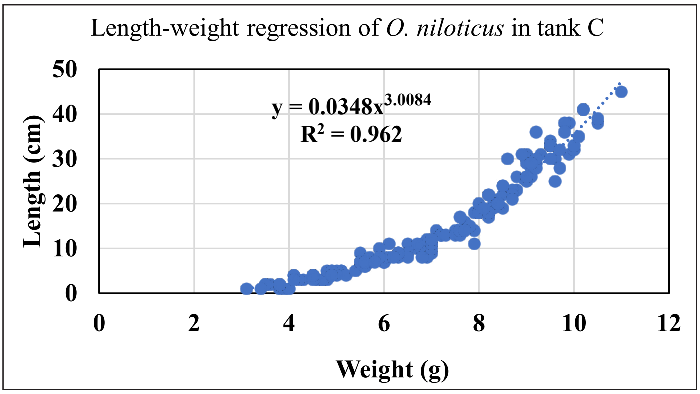

Figure 11. Length-weight regression of *Oreochromis niloticus* in Tank C (aerator) shows isometric growth (b=3)

Survival and Death Rate, Weight Gained, Specific Growth Rate and Feed Conversion Ratio of *Oreochromis niloticus*

Table 3 shows the survival rate (SR%), weight gain (WG%), specific growth rate (SGR%), feed conversion ratio (FCR%) and death rate (MR%) of *O. niloticus* in Tank A, Tank B, and Tank C. *Oreochromis niloticus* in both Tank A and Tank C show the highest SR% (100.00 ± 0.00) and 0.00% for MR%, respectively. Differently in Tank B, the lowest SR% (98.00 ± 1.41) and highest MR% (2.00%) were recorded as one of the fish unable to survive during the experiment. In the present study, the best FCR% was in Tank C with 6.50 ± 5.88, and the lowest was in Tank B (11.20 ± 6.92). WG% of *O. niloticus* in Tank A (120.54 ± 0.61), tank B (71.67 ± 1.07) and Tank C (140.63 ± 5.23) showed significant differences ($p>0.05$) and were separated into different groups. WG% was highest in Tank C in the present study, with the lowest FCR%. The specific growth rate (SGR%) measures the daily percentage increase in fish weight (Lugert et al., 2016). The SGR% recorded was 1.54 ± 0.27 in Tank A, 2.41 ± 0.04 in Tank B, and the highest at 4.82 ± 0.37 in Tank C. A significant difference was observed between the treatments and the control.

The values, n = 65, are given as mean + standard deviation. In a column, different superscript letters ^{a,b,c} denote significant differences across treatments ($p<0.05$).

Table 3
Oreochromis niloticus *survival rate, mortality rate, weight gained, specific growth rates and feed conversion ratio in Tank A (control), Tank B (*E. densa*) and Tank C (O_2 pump)*

Parameter	**Treatments**		
	Tank A (control)	**Tank B (*E. densa*)**	**Tank C (O_2 pump)**
SR%	100.00 ± 0.00^{a}	98.00 ± 1.41^{a}	100 ± 0.00^{a}
WG%	120.54 ± 0.6^{b}	71.67 ± 1.07^{c}	140.63 ± 5.23^{a}
SGR%	2.41 ± 0.04^{b}	1.54 ± 0.27^{c}	4.82 ± 0.37^{a}
FCR%	8.00 ± 2.42^{b}	11.20 ± 6.92^{c}	6.50 ± 5.88^{a}
MR%	0.00%^{a}	2.00%^{a}	0.00%^{a}

Note. SR% = survival rate; WG%= weight gained; SGR% = specific growth rates; FCR% = feed conversion ratio; MR% = mortality rate; O_2 = oxygen; *E. densa = Egeria densa*

DISCUSSION

Effects of Dissolved Oxygen towards the Growth of *Oreochromis niloticus*

DO is crucial in aquaculture as fish absorb oxygen passively through their gills (Valverde et al., 2006). It comes from air diffusion and photosynthesis by chlorophyll-containing organisms (Abdel-Tawwab et al., 2015). Oxygen transfer depends on a concentration gradient, requiring adequate DO levels (Yildiz et al., 2017).

Limitations of this study include the potential for *E. densa* to introduce confounding factors beyond oxygenation, as the plant may compete with fish for available nutrients, potentially affecting growth and feeding performance. Nighttime measurement of DO levels also could not be carried out due to safety concerns at the study site.

Dissolved Oxygen was monitored three times per week at different times, which are during morning, midday, and afternoon. The recorded values were averaged before being analysed. DO concentrations ranged from 8.82 to 12.32 mg/L, which appears to fall within the typical range. Healthy water typically has DO above 5.0 mg/L (Rouf et al., 2022), while levels below 1-2 mg/L can be fatal (Rouf et al., 2022). Fish growth declines when DO is between 3-5 mg/L (Stickney, 2017). If DO drops below 3 mg/L, feeding should stop, and corrective actions should be taken. Tilapia require at least 2.0 mg/L for smaller fish (60-100g) and 5.0 mg/L for larger fish (200-270g) (Saravanan et al., 2012). Atlantic salmon, however, cannot survive in less than 6 mg/L, as this is considered hypoxic (Alver et al., 2022).

This study confirms that oxygen is a crucial abiotic factor affecting fish development, including *O. niloticus*, where low DO levels negatively impact length and weight. Higher DO levels in Tank C likely contributed to better fish growth, supporting findings from Abdel-Tawwab et al. (2015). This is further validated by the length-weight regression analysis as the exponent b value indicates growth patterns with $b<3$ negative allometric growth (fish grow longer but remain lean), $b=3$ isometric growth (length and weight increase proportionally), and $b>3$ signifies positive allometric growth (fish become stouter as they grow) (García-Fernández et al., 2020).

Lentic waters with low DO impede fish growth. *O. niloticus* in Tank C showed isometric growth ($b=3.0084$, $b>3$), proving that increased DO from aeration (3.2 mg/L) boosted growth compared to tank A. Lakani et al. (2013) also observed lower body weight in fish reared in normal and low oxygen conditions compared to those in high oxygen environments. Comparably, halibut *(Hippoglossus hippoglossus* L.) growth gradually increased as oxygen saturation rose from 57% to 100% (Thorarensen et al., 2010). Following the cessation of oxygen supply to Tank C's water pump, growth rate declined, aligning with Franklin (2014), who asserts that DO is influenced by the re-aeration, which is a key factor in fish habitat quality and stream health. Other studies show comparable findings, such as channel catfish, *Ictalurus punctatus* (Buentello et al., 2000), spotted wolfish, *Anarhichas minor* (Foss et al., 2002), striped bass, *Morone saxatilis* (Brandt et al., 2009), Atlantic halibut, *Hippoglossus hippoglossus* (Thorarensen et al., 2010), Japanese flounder, *Paralichthys olivaceus* (Duan et al., 2011), grass carp, *Ctenopharyngodon idella* (Gan et al., 2013) and Nile tilapia, *O. niloticus* (Abdel-Tawwab et al., 2014).

DO levels are regulated by production (photosynthesis) and consumption (respiration, organic matter breakdown, and oxygen demand) (Banerjee et al., 2019).

Goodwin et al. (2008) stated DO is produced during the day through photosynthesis but continuously consumed by respiration, causing fluctuations. Despite *E. densa* supplying oxygen in Tank B, fish growth remained negative allometric. This is due to competition for DO between *O. niloticus* and *E. densa,* especially during nighttime when oxygen levels decline under dense macrophyte cover, resulting in hypoxic conditions (Ribaudo et al., 2014). According to Białowiec et al. (2019), *E. densa* increased oxygen levels during daylight and then shifted to oxygen consumption during the night due to respiration. The respiratory activity of *O. niloticus* and *E. densa* at night likely contributed to a significant reduction in DO levels in Tank B, which may have negatively affected the growth performance.

Fish in Tank A (control) had significantly lower growth than those in Tank C. Lotic waters (streams, rivers) dissolve more oxygen than lentic waters (ponds, lakes) (McDonald, 2012). The lentic condition in Tank A reduced DO levels, limiting *O. niloticus* growth. As a result, fish in this tank exhibited negative allometric growth due to insufficient oxygen for movement, feeding, and metabolism. As fish grow, their oxygen demand increases faster than their ability to absorb oxygen, leading to abnormal growth due to the disproportionate expansion of body volume over surface area (Machado, 2019). This study supports findings by Mallya (2007), which reported that low DO levels reduce food intake and decelerate fish growth. When DO declines, fish exhibit aquatic surface respiration (ASR) where they swim to the surface for oxygen-rich water (Franklin, 2014). This behaviour was often observed in Tanks A and B.

Comparing the effects of additional DO and natural DO shows that both significantly impact fish growth. These findings confirm previous studies suggesting that optimal growth occurs at oxygen saturation levels of around 100% or greater (Domenici et al., 2017). In this study, fish in the control tank without an oxygen supply were significantly smaller in size than those in the hyperoxia. Poor growth under low DO conditions is likely due to inadequate oxygen availability for crucial metabolic processes.

Effects of Dissolved Oxygen on the Survivability of *Oreochromis niloticus*

DO is crucial for fish growth, and consistently low levels can reduce feed intake, growth, and feed efficiency (Li et al., 2020). Most post-larval fish absorb oxygen through their gills (Cox, 2003). When DO drops below the required threshold, fish strive to convert energy efficiently, leading to slower growth, feeble swimming, and lower feed efficiency (Roslan et al., 2021). In response, fish intensify opercular ventilation and gasp for air. Abdel-Tawwab et al. (2014) found that low DO significantly slowed Nile tilapia growth by reducing feed intake due to poor appetite and digestion.

This experiment examined DO's impact on *Oreochromis niloticus* survival rate (SR%) and feed conversion efficiency (FCR%). Tanks A and C had the highest SR% with no recorded deaths, while Tank B had the lowest SR% and highest mortality rate (MR%). Deaths in Tank B occurred due to fish caught up in *E. densa*, preventing feeding and depleting energy. The struggle to escape increased metabolism and exhaustion of the fish. Maharani et al. (2022) noted that survival rates are influenced by feeding frequency, feed-to-weight ratio, and feed quality, which indirectly affected the SR% in this study. Therefore, DO may not be the key factor impacting *O. niloticus* survival in this experiment.

Survival rate measures the percentage of organisms remaining alive over time, affected by water quality, feed and proper maintenance. Ahmed et al. (2013) recorded Tilapia survival rates of 82-90%, aligning with this study. Mortality rate, which includes natural and fishing mortality, reflects fish population loss (Abu El-Nasr, 2021). Natural mortality results from predation, disease, pollution, or environmental factors (Allen & Hightower, 2010), while fishing mortality happens due to being caught by various gear (Sparre et al., 1989).

According to the United States Agency for International Development (2011), the feed conversion ratio (FCR) measures the amount of feed required for fish growth. A lower FCR% indicates better feed efficiency and a diet quality (Iskandar & Elrifadah, 2015; USAID, 2011). Adequate DO spikes fish activity and digestion, resulting in higher feed intake and more efficient growth. Mengistu et al. (2019) reported a positive correlation between feeding rate and DO, indicating that increasing DO from the lowest to the highest levels improved FCR by 50%. Tank C had the lowest FCR%, suggesting optimal water quality for feeding, while Tank B showed the highest FCR%. However, the large standard deviation probably indicates variations in growth among individuals and feed intake. The mean FCR still represents the treatment's feed conversion efficiency. Elvy et al. (2022) noted that a lower FCR% is linked to reduced energy needs for digestion. Research by Abdel-Tawwab et al. (2015), Bergheim et al. (2006), and Duan et al. (2011) confirmed that oxygen availability and diet affect fish growth rate and feed efficiency.

FCR%, WG%, and SGR% are closely related, as noted by Brandt et al. (2009) and Duan et al. (2011). Specific growth rate (SGR%) measures the daily weight gain percentage of fish (Lugert et al., 2016). Higher WG% and SGR% in a hyperoxic environment suggest increased feed intake due to better digestion and nutrient absorption (Gan et al., 2013; Tran-Duy et al., 2008). In contrast, fish in low DO conditions consumed less feed, had the highest FCR, and showed poor growth. Adequate DO assists fish in reducing energy expenditure, permitting more energy for growth and fat storage (Duan et al., 2011). Growth rate is also influenced by supplemental feeding, sex, stocking density, and water temperature (Mizanur et al., 2014). This study also demonstrated a correlation between SGR% and WG%, with Tank C displaying the highest and Tank B the lowest values for both parameters.

In our opinion, SGR% was lowest in Tank B despite the presence of *E. densa*, which provides oxygen through photosynthesis. By referring to a previous study, at night, *E. densa* consumes oxygen through respiration, leading to DO depletion.

Rodrigues and Thomas (2010) revealed that *E. densa* induces oxygen deficiency from 2.4 to 16.9 mg/L due to dark respiration, concurring with this study. High DO consumption at night created competition with *O. niloticus*, inducing severe hypoxia stress affecting fish metabolism. Duan et al. (2011) suggested that fish in low DO conditions use energy to maintain metabolism, reducing lipid and protein levels. Similarly, Abdel-Tawwab et al. (2015) found that fish in low DO had lower protein and fat levels. Duan et al. (2011) also reported that fish in well-oxygenated water exhibit better feed efficiency and growth performance.

The Effectiveness of *E. densa* as a Natural Oxygenator to Replace Oxygen Pumps in Aquaculture

Environmental hypoxia naturally occurs in aquatic ecosystems when oxygen consumption by plants, bacteria, and animals during the dark cycle exceeds oxygen production or diffusion from the atmosphere (Richard, 2011). Prolonged hypoxia can disrupt respiration, reduce metabolic rates and feed intake, hinder growth, and increase susceptibility to disease (Li et al., 2020). Dissolved oxygen (DO) is therefore critical for freshwater fish, invertebrates, plants, and microbial communities, as it supplies the free oxygen required for survival. Without sufficient DO, most aquatic organisms are unable to endure anoxic or hypoxic conditions for extended periods (Mitchell-Holland et al., 2018).

Growth observations of *Tilapia* sp. demonstrate that fish reared in oxygen-pumped tanks exhibit isometric growth, whereas those in control and *E. densa* tanks show negative allometric growth, highlighting the importance of adequate oxygen supply. Although *E. densa* produces oxygen via photosynthesis, it is unsuitable as a substitute for mechanical oxygenation. This species thrives in diverse aquatic environments due to its ability to utilise bicarbonate as an inorganic carbon source and tolerate low-light conditions; however, it consumes oxygen during the night to aid respiration and growth (Rodrigues & Thomaz, 2010).

Rodrigues and Thomaz (2010) reported substantial oxygen depletion caused by *E. densa* dark respiration, ranging from 2.4 to 16.9 mg/L. These findings support Piria et al. (2022) and contradict Yarrow et al. (2009). Previous studies have presented that DO concentrations typically rise during daylight hours, peak in the afternoon, and fall at night, hitting their lowest levels around early morning (Oldham et al., 2018; Ribaudo et al., 2014). In still or lentic water bodies with dense macrophyte coverage, nighttime hypoxia is common as oxygen consumption surpasses replenishment through atmospheric diffusion, particularly near the benthic zone (Ersoy et al., 2020). This indicates that submerged macrophytes are unsuitable as primary oxygen sources in lentic systems.

Although *E. densa* serves as an effective carbon sink, storing carbon at rates comparable to seagrasses and tidal marshes (Drexler et al., 2021), its role as an oxygenator remains limited. Few studies have examined oxygen exchange constraints during the dark respiration of submerged macrophytes. Mitchell-Holland et al. (2018) reported that *E. densa* is ineffective as an oxygen supplier, whereas *Ceratophyllum demersum* demonstrates greater potential as an oxygenating species. While the utilisation of aquatic plants as biological catalysts for DO enhancement could reduce reliance on energy-intensive oxygen pumps and lower operational and management costs, the present evidence suggests that *E. densa* is unsuitable for this role. Consequently, the aquarium and water garden industries should promote more effective native macrophytes, such as *C. demersum*, to improve water quality and aquatic habitat conditions.

CONCLUSION

Oxygen is crucial for *O. niloticus* as it affects its growth, survival, and feeding. DO levels directly influence the fish's digestion and metabolism. Low oxygen reduces fish quality, resulting in smaller growth, which increases the number of fish needed per kilogram in aquaculture, indirectly impacting agricultural and fishing income. Furthermore, *E. densa* demonstrates marginal effectiveness as a natural oxygenator in aquaculture systems compared to an oxygen pump, as its rate of oxygen production is incompatible with that produced by the pump. Further studies on high dissolved oxygen conditions in aquaculture are required to provide clearer evidence on their effects on cultured fish. Future research should include continuous DO monitoring and better control of confounding factors to isolate hyperoxic effects.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR'S CONTRIBUTION STATEMENTS

NHR executed and supervised the research, SNK collected data and prepared the manuscript, AZMZ, NAR and SSZ conceived the idea, NAO and SMS handled analysis, and NAY and SNLR edited the manuscript.

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