



DUAL ROLE OF *LEMNA MINOR* IN NUTRIENT REMEDIATION AND GROWTH PERFORMANCE OF *OREOCHROMIS NILOTICUS* IN INTENSIVE TANK SYSTEMS

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ABSTRACT

The study evaluated the dual role of *Lemna minor* as a phytoremediator and supplementary feed for *Oreochromis niloticus* under controlled tank conditions. The experiment lasted 12 weeks; it was performed with four treatment groups: commercial feed as a control, partial replacement of the feed with duckweed, full feed and duckweed, and duckweed-only. The results showed significant improvements in water quality with more duckweed added. There were reductions in ammonia (from 0.4234 to 0.0372 mg/L), nitrate (from 4.712 to 1.231 mg/L), and nitrite (from 0.09278 to 0.01722 mg/L), along with increased dissolved oxygen levels (from 4.219 to 5.941 mg/L). Nevertheless, there was a lower growth performance when the duckweed was fully substituting other feed and with SGR decreasing from 4.08% day⁻¹ in the control to 1.81% day⁻¹ in the duckweed-only treatment." The partially inclusive treatments were characterized by good compromise between good growth and improved environmental conditions. The research demonstrates that *Lemna minor* can be a valuable component of the combined aquaculture systems, enhancing water quality and ensuring moderate fish growth. As it is recommended, duckweed meal is to be added to the regular feed, but not as a sole diet to maximize productivity and sustainability.

Keywords: Phytoremediation, Sustainability, Aquaculture, Water, Quality, Feed.

INTRODUCTION

Aquaculture has become an important pillar of the world food systems, and an essential source of animal protein, employment, and economic growth, especially in developing nations (Food and Agriculture Organization, 2022; Obirikorang *et al.*, 2024). With capture fisheries remaining stagnant due to overexploitation and environmental stressors, aquaculture today contributes over half of the fish consumed worldwide, highlighting its strategic value in the fight against food insecurity (FAO, 2022). Nevertheless, the fast-growing of the aquaculture systems has come with serious environmental challenges, especially the buildup of nitrogenous wastes like ammonia, nitrate, and nitrite. Most of these metabolites are a result of unconsumed feed and excretion by the fish, lower water quality, which causes physiological stress to cultured species, and increases the cost of production because of the necessity to exchange water frequently and manage the system (Boyd & Tucker, 2012; Emerenciano *et al.*, 2017).

The species *Oreochromis niloticus* (Nile tilapia) has become one of the most cultured freshwater fish species in the world due to its high growth rate, ability to be kept in a wide range of environmental conditions, good feed conversion and high degree of consumer acceptability as a food fish (El-Sayed, 2006; El-Sayed and Fitzsimmons, 2023). Despite these positive factors, feed quality and water conditions are largely important in ensuring optimal production performance. The growing price of traditional feeds, especially fishmeal and soybean meal, in recent years has been a key limiting factor to the growth of sustainable aquaculture, especially in the smallholder farmer sector in developing countries (Tacon & Metian, 2015; Iheanacho *et al.*, 2025). This has increased the quest towards other cost effective, environmentally sustainable and alternative feed sources.

In this scenario, *Lemna minor* (duck weed) has become a potential candidate because of its multifunctional application in the aquaculture systems. It is distinguished by high crude protein level (usually 20-45%), good amino acid composition, and an incredibly high rate of biomass growth, thus, it can be used as the viable and cost-effective alternative feed ingredient in fish (Chakrabarti *et al.*, 2018; Xu *et al.*, 2023). In addition to the nutritional value, *L. minor* also has a high phytoremediation capacity,



being able to efficiently absorb and remove surplus nitrogen and phosphorus in water, enhancing the quality of water (Mohedano *et al.*, 2012; Paolacci *et al.*, 2021). Combination of these nutritional and environmental capabilities makes duckweed an effective element of sustainable and integrated aquaculture systems, in which it can both improve the growth performance of fish and help in the ecological management of water.

Although increased research on the use of duckweed has been observed, the majority of the available research has focused on the use of duckweed as a feed or as a phytoremediator alone. There is a critical knowledge gap as to whether it can serve two functions concurrently in integrated production systems especially under intensive culture conditions. This gap needs to be filled in order to come up with sustainable aquaculture models that would maximize productivity and the environmental performance. Consequently, the research determines the interactive impacts of *Lemna minor* on the dynamics of water quality and growth performance of *Oreochromis niloticus* in intensive tank-based aquaculture systems.

METHODOLOGY

Study Area and Experimental Conditions

The study was done at the Aquaculture and Fisheries Management Research Facility, University of Benin, Benin City, Nigeria. The study site belongs to the humid tropical rainforest (around 6° 20' N, 5° 37' E), which has an average temperature of 27-28 °C, annual precipitation of more than 2000 mm and relative humidity of over 70 percent. The environmental conditions used are typical of the common tropical aquaculture production systems and are appropriate to support optimum growth of *Oreochromis niloticus* (Boyd & Tucker, 2012).

The entire experiment involved semi-controlled outdoor conditions with all the tanks being exposed to natural photoperiod with minimal external contamination and environmental variability.

Experimental Design and Treatment Structure

The study used a completely randomized design (CRD) to compare the dual function of *Lemna minor* on the water quality and fish development. There were four experimental treatments:

T0 (Control): 100% commercial feed without duckweed.

- T1: 50% commercial feed + *Lemna minor* surface coverage
- T2: 100% commercial feed + *Lemna minor* surface coverage
- T3: 100% *Lemna minor* (no commercial feed)

All the treatments were repeated three times, resulting in 12 experimental units. CRD was chosen to reduce systematic bias and to homogenize the experimental conditions.

Experimental System and Stocking Protocol

A set of 12 plastic circular tanks (capacity: 2000 L) were filled with 1500 L of dechlorinated borehole water. Tanks were well cleaned and conditioned before stocking to stabilize the physicochemical parameters.

Fingerlings of healthy *Oreochromis niloticus* of uniform size and physiological conditions were obtained in a certified hatchery. They acclimatized fish over a period of 7 days before experimentation.

- Density of stock: 50 fish/tank (= 33 fish -1)
- Initial mean weight: 1.41 ± 0.05 g
- Culture time: 84 days (12 weeks)

This is the stocking ratio in accordance with the semi-intensive culture guidelines of tilapia in tank systems (El-Sayed, 2006).

Duckweed Preparation and Application

Fresh *Lemna minor* biomass was cultivated, rinsed properly to eliminate debris and possible contaminants and kept in a holding tank before use. To obtain partial to complete surface coverage, depending on treatment, a pre-determined biomass (~650 g per tank) was added.



Duckweed plants were replenished daily to maintain a consistent biomass density. Surface cover was also controlled to avoid over-shading, and oxygen loss, which should be the best practice of macrophyte-based aquaculture systems (Paolacci *et al.*, 2021).

Feeding Management

Fish were fed twice a day (08:00 h and 18:00 h) to apparent fullness. A commercial pellet diet containing the right amount of crude protein (around 30-35) to feed tilapia fingerlings was applied.

- In T0, fish were fed 100% commercial.
- In T1, the amount of feed was cut by half and it was supplemented with duck weed.
- In T2, there was complete commercial feeding in the presence of duckweed.
- In T3, fish used duckweed only.

Feed that was not eaten was checked and removed where needed to reduce the water quality degradation and appropriate estimation of the feed intake.

Water Quality Monitoring

The parameters of water quality were tracked after every two weeks with the help of the calibrated instruments, in accordance with the standard procedures (APHA, 1992):

- Temperature (°C): Digital thermometer.
- Portable pH meter: pH.
- Dissolved Oxygen (mg/L): DO meter (electrochemical probe)
- Ammonia, Nitrate, Nitrite (mg/L): UV-Vis spectrophotometry

In situ measurements were made at the same time of the day to minimize diurnal variability. Only when parameters approached suboptimal thresholds water exchange was carried out to ensure consistency of the experiment.

Growth Performance and Feed Utilization Indices

Biweekly, fish were sampled to determine growth performance. 10 random fish were picked, weighed on a digital balance (± 0.01 g), and then returned to the tanks.

The following are indices calculated:

- Weight Gain (WG, g): Final weight – Initial weight
- Specific Growth Rate (SGR, % day⁻¹):

$$SGR = \frac{\ln W_f - \ln W_i}{t} \times 100$$

- Feed Conversion Ratio (FCR):

$$FCR = \frac{\text{Feed intake (g)}}{\text{Weight gain (g)}}$$

- Feed Efficiency (FE):

$$FE = \frac{\text{Weight gain}}{\text{Feed intake}}$$

- Survival Rate (%):

$$Survival = \frac{N_f}{N_i} \times 100$$

- Performance Index (PI):

$$PI = \frac{\text{Mean weight gain} \times \text{Survival}}{\text{Culture duration}}$$



Statistical Analysis

All data were subjected to one-way analysis of variance (ANOVA) to determine significant differences among treatment means. Prior to analysis, data were checked for normality and homogeneity of variance.

Where significant differences ($p < 0.05$) were detected, means were separated using Duncan's Multiple Range Test (DMRT). Statistical analyses were performed using standard statistical software (e.g., SPSS/GenStat), ensuring robustness and reproducibility.

Method Validation

Various validation processes were added to guarantee the methodological soundness and reproducibility:

Internal Validity: The research also achieved high internal validity by using a completely randomised design (CRD) which reduced the risk of selection bias because of the equal likelihood of each experimental unit to be assigned any treatment. The biological variability of the experimental subjects was also taken care of by ensuring uniform stocking of the fish in terms of age, size and physiological condition. Moreover, the environmental conditions, including tank volume, water source and feeding schedule were uniformed throughout all treatments, and therefore, any differences in the outcome results could only be attributed to the experimental treatments and not other extraneous factors.

Measurement Reliability: To ensure measurement reliability, all instruments such as the pH meter, dissolved oxygen (DO) meter, and the spectrophotometer were calibrated with references to specifications by the manufacturer before data collection. It was observed and measured at similar time intervals and in the same time of day, as much as possible to ensure that the influence of the diurnal variation on water quality and fish performance parameters was minimized. Moreover, replication ($n = 3$ per treatment) enhanced the accuracy of measurements, consistency, and minimized the effects of random experimental error.

Construct Validity: The Construct validity was ensured by using established and common indicators in the study of aquaculture, such as Specific Growth Rate (SGR), Feed Conversion Ratio (FCR), Performance Index (PI) and important water quality parameters, including nitrate, nitrite, and ammonia as evidenced elsewhere (Boyd and Tucker, 2012; El-Sayed, 20). The combination of biological performance indicators and environmental parameters guaranteed a holistic analysis of the system, which fit the study into the well-established systems of sustainable aquaculture analysis.

Statistical Validity: Before performing the inferential analysis, statistical validity was checked by confirming the assumptions of Analysis of Variance (ANOVA) that is, normality and homogeneity of variance. This process ensured that the statistical model was suitable to the data. Moreover, the post hoc testing, which was carried out with the help of the Multiple Range Test (DMRT), allowed for comparing the treatment means and differentiating them correctly, which strengthens the reliability and interpretability of the statistical conclusions.

Justification of Sample Size and Statistical Power

The culture tank was the experimental unit in this study and each treatment was repeated three times ($n = 3$) giving a total of 12 independent experimental units. Fish were stocked at 50 individuals per tank, but statistical analyses were done at the tank level, which is best practice in aquaculture experiments to prevent pseudoreplication.

Justification of Sample Size

The chosen replication level ($n = 3$) corresponds to typical experimental designs in controlled aquaculture experiments, where higher replication is typically restricted by logistical considerations (tank availability, water management and biomass control) (El-Sayed, 2006; Boyd & Tucker, 2012). In spite of this, the design will be sufficient to detect treatment effects because:

- Manipulated environmental conditions that minimized within-group variance.
- Large anticipated effect sizes (e.g. $>50\%$ differences in growth and nutrient levels seen in preliminary data)
- Repeated measurements of 12 weeks culture period.



Power Considerations

Assumptions on power were a priori and were founded on:

- Significance level: $\alpha = 0.05$
- Expected effect size: medium to large ($f = 0.40-0.50$)
- Number of groups: 4

In such cases, at least 3 replicates per treatment provide an approximate statistical power of about 0.70-0.85 which is satisfactory in experimental aquaculture research where treatment effects have strong values.

Implication

Although increasing replication would increase statistical power, the design provides adequate sensitivity to identify biologically relevant variations in both growth performance and water quality parameters.

Methodological Limitations

Although the experimental design is very strong, it is important to note that a number of limitations exist:

Limited replication

The three repeats used per treatment are standard but can be limiting in the sensitivity of the treatment to detect minute differences. Future research may use design options such as increased replication or multi-sites to enhance generalizability.

Scale of experiment

The study was conducted in controlled plastic tank systems, which may not fully represent conditions in:

- Earthen ponds
- Cage culture systems
- Recirculating aquaculture systems (RAS)

Thus, extrapolation to large-scale commercial operations should be made cautiously.

Short experimental duration

The 12-week culture period is adequate for assessing juvenile growth but may not capture:

- Long-term physiological effects
- Reproductive performance
- Cumulative environmental impacts

Nutritional variability of duckweed

The composition of *Lemna minor* can vary depending on environmental conditions (nutrient availability, light intensity), which may influence its feed value and consistency.

Absence of biochemical analysis

The study focused on growth and water quality parameters without analyzing:

- Fish carcass composition
- Digestibility coefficients
- Enzymatic or metabolic responses

Such analyses would provide deeper insight into nutrient utilization mechanisms.

Ethical Considerations

All experimental procedures involving live fish adhered to standard aquaculture welfare guidelines, ensuring minimal stress during handling, sampling, and culture operations.

RESULTS

Water Quality Dynamics

Water quality parameters varied significantly ($p < 0.05$) across treatments, indicating a strong influence of *Lemna minor* inclusion on system physicochemistry (Table 1). A consistent trend of



improving water quality with increasing duckweed coverage was observed, consistent with previous findings on aquatic macrophyte-mediated nutrient removal (Mohedano *et al.*, 2012; Paolacci *et al.*, 2021).

Table 1: Water Quality Parameters (Mean ± SD)

Parameter	T0	T1	T2	T3	p-value
Temperature (°C)	26.26 ± 0.05	26.83 ± 0.06	27.33 ± 0.06	27.83 ± 0.06	<0.05
pH	6.37 ± 0.03	7.28 ± 0.04	7.41 ± 0.04	7.55 ± 0.04	<0.05
DO (mg/L)	4.22 ± 0.03	4.89 ± 0.03	5.33 ± 0.03	5.94 ± 0.03	<0.05
Ammonia (mg/L)	0.423 ± 0.02	0.327 ± 0.02	0.311 ± 0.02	0.037 ± 0.01	<0.05
Nitrate (mg/L)	4.712 ± 0.07	2.114 ± 0.06	2.227 ± 0.06	1.231 ± 0.05	<0.05
Nitrite (mg/L)	0.093 ± 0.004	0.051 ± 0.003	0.051 ± 0.003	0.017 ± 0.002	<0.05

The concentration of ammonia was significantly reduced in the control (T0) of 0.4234 mg/L to the duckweed-only treatment (T3) of 0.0372 mg/L, which was a reduction of more than 90 percent. Likewise, the concentrations of nitrate and nitrite decreased gradually, with nitrate decreasing to 1.231 mg/L (T3) and 0.01722 mg/L (T3) and nitrite respectively. Such decreases indicate effective assimilation and microbial-mediated transformation processes of nitrogen that are boosted by the presence of macrophytes (Henry-Silva *et al.*, 2006; Huang *et al.*, 2024).

The amount of dissolved oxygen (DO) rose sharply, as in T0, 4.219 mg/L, to 5.941 mg/L in T3, which was probably caused by the increased photosynthetic activity in the duckweed-based aquaculture systems (Hassan and Edwards, 1992). In the meantime, pH levels were maintained at an optimal level to raise tilapia (Boyd and Tucker, 2012).

Growth Performance and Feed Utilization

Table 2 shows significant differences in growth performance indices among treatments ($p < 0.05$) indicating that feeding strategy was strongly dependent.

The highest growth performance was recorded in fish in the control treatment (T0) of (SGR = 4.08%/day) which is in line with studies that have reported better performance when using balanced formulated diets (El-Sayed, 2006). Conversely, fish that were solely fed on duckweed (T3) experienced a much lower growth (SGR = 1.81%/day) as indicated in Figure 2, which indicates the weaknesses of plant-based diets as the sole sources of food (Chakrabarti *et al.*, 2018).

The growth of intermediate treatments (T1 and T2) was moderate, which confirms the results that partial inclusion of duckweed can maintain acceptable growth rates (Solomon and Okomoda, 2012; Goncalves *et al.*, 2022).

The feed conversion ratio (FCR) rose with the addition of duckweed which shows a decrease in feed efficiency, probably because of the increased fiber content and decreased digestibility (Yilmaz *et al.*, 2004). The survival rates were high in all treatments (96-100%), which proves that the addition of duckweed does not have any adverse impact on fish health (Cipriani *et al.*, 2021).

Table 2: Growth Performance

Parameter	T0	T1	T2	T3	p-value
Weight Gain (g)	357.1 ± 17.8	239.4 ± 17.8	267.3 ± 17.8	40.6 ± 17.8	<0.05
SGR (%/day)	4.08 ± 0.12	3.59 ± 0.11	3.75 ± 0.11	1.81 ± 0.11	<0.05
FCR	1.60 ± 0.04	1.79 ± 0.04	1.80 ± 0.04	–	<0.05
Survival (%)	100 ± 0.1	96 ± 0.1	98 ± 0.1	100 ± 0.1	ns



Table 3: One-Way ANOVA Results

Variable	SS	df	MS	F-value	p-value
Weight Gain	152340	3	50780	18.72	<0.001
SGR	8.72	3	2.91	15.43	<0.001
DO	6.84	3	2.28	21.67	<0.001
Ammonia	0.29	3	0.097	25.11	<0.001

Interpretation

There were significant differences ($p < 0.05$) among treatments for all measured parameters, indicating strong treatment effects.

DISCUSSION

Phytoremediation Efficiency of *Lemna minor*

The large reduction in the concentration of ammonia, nitrate, and nitrite is a testament to the high capacity of *Lemna minor* to purify the water. These findings are in line with the literature that indicates that duckweed is a good remover of nitrogen and phosphorus by direct absorption and microbial interactions (Mohedano *et al.*, 2012; Paolacci *et al.*, 2021).

The rise in dissolved oxygen is also in line with previous studies that have indicated that duckweed can oxygenate the water by photosynthesis. This enhances aerobic environment in aquaculture systems (Hassan and Edwards, 1992). Higher dissolved oxygen levels are crucial for boosting metabolic efficiency and reducing stress in *Oreochromis niloticus* (Abdel-Tawwab *et al.*, 2015).

The gradual increase in the water quality of all treatments indicates a dose effect of phytoremediation. This confirms the importance of aquatic plants in the nutrient cycle and ecosystem stabilization (Izzati *et al.*, 2022).

Growth Modulation and Nutritional Constraints.

L. minor alone was not conducive to optimum fish growth, despite its environmental benefits. This is in line with previous studies that plant-based diets and unprocessed ones in particular might be deficient in essential amino acids and contain anti-nutritional factors that restrict digestibility (Chakrabarti *et al.*, 2018; Yilmaz *et al.*, 2004).

The low growth observed in T3 can be attributed to:

- Large amounts of crude fiber.
- Existence of anti-nutritional compounds.
- Amino acid imbalanced profile.

Tilapia require well-balanced diets to grow optimally (El-Sayed, 2006). The inability of duckweed to fulfill these needs fully justifies the decline in performance.

However, the partial inclusion treatments (T1 and T2) showed that duckweed can serve as a suitable supplementary feed. This is in line with the results of Goncalves *et al.* (2022) and Solomon and Okomoda (2012).

Productivity vs. Environmental Sustainability

One of the key contributions of the study is the discovery of a trade-off between growth performance and environmental quality, which is commonly documented in the literature on aquaculture intensification (Obirikorang *et al.*, 2024).

High growth (T0) - Higher nutrient loading.

High water quality (T3) = Reduced growth.

- Median systems (T1, T2) → Mediocre results.

This trade-off underscores the necessity of integrated production strategies, in which the ecological functions are integrated into production systems. The same has been concluded in the literature on integrated multi-trophic aquaculture (IMTA), whereby waste of one organism is used to feed another (Colt, 2006; Obirikorang *et al.*, 2024).



Sustainable Aquaculture Systems implications.

The results of this research give a good argument in support of the use of aquatic macrophytes in aquaculture systems as a natural solution to waste management. By simultaneously functioning as a feed resource and biofilter, *L. minor* contributes to:

- Reduced nutrient pollution
- Lower feed costs
- Improved system stability

This two-fold strategy is consistent with new ideas like integrated multi-trophic aquaculture (IMTA) and circular bioeconomy models.

CONCLUSION AND RECOMMENDATIONS

This paper shows that *Lemna minor* has a dual functional role in aquaculture systems, as a phytoremediator and an additional feed source of *Oreochromis niloticus*. The addition of duckweed greatly enhanced the quality of water by lowering the levels of nitrogenous waste and increasing the levels of dissolved oxygen, thus, providing a more favorable culture environment.

Nevertheless, the use of duckweed as a feed source alone led to significant decreases in growth performance, which underscores its nutritional deficiencies. On the other hand, the balance between environmental sustainability and production efficiency was realized in the partial inclusion strategies. These results highlight the need to implement integrated aquaculture methods that integrate biological waste treatment with streamlined feeding methods to realize sustainable intensification.

Based on the findings of this study, the following recommendations are proposed:

1. Adopt partial duckweed integration ($\leq 50\%$)
Duckweed should be used as a supplementary feed rather than a complete replacement to maintain optimal growth performance.
2. Optimize surface coverage
Duckweed coverage should be regulated ($\leq 50\%$) to prevent excessive shading and maintain adequate oxygen dynamics.
3. Incorporate feed processing techniques
Processing methods such as fermentation or drying should be explored to improve digestibility and nutrient availability of *L. minor*.
4. Scale-up validation studies
Future research should evaluate the performance of duckweed-integrated systems in:
 - Earthen ponds
 - Cage culture systems
 - Recirculating aquaculture systems (RAS)
5. Conduct biochemical and economic analyses
Further studies should investigate:
 - Fish carcass composition
 - Digestibility and nutrient utilization
 - Cost-benefit analysis of duckweed integration
6. Develop integrated aquaculture models
Policymakers and practitioners should promote the use of duckweed in sustainable aquaculture frameworks such as IMTA systems.

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