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Effect of Light Intensity on Ammonium Removal and Biomass Growth in Different Levels of Aquaculture Effluent Using Duckweed (*Lemna perpusilla*)

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Abstract: Cultivating duckweed in aquaculture effluent offers a viable approach to eliminating contaminants. The duckweed biomass obtained can be utilized for the generation of bioenergy. However, elevated ammonium (NH4+) levels in aquaculture effluent, combined with variations in light intensity, can hinder biomass formation. The precise mechanisms underlying this inhibition remain incompletely elucidated. The study assessed the efficacy of duckweed (*Lemna perpusilla)* as a treatment agent for wastewater from catfish farms. The objective was to evaluate the growth response of duckweed and its efficacy in reducing ammonium levels. The research demonstrated that daily light intensity fluctuated using shade nets and that the ammonium concentration of aquaculture wastewater varied according to the age of the fish. The shade nets, which blocked 25% of the sunlight and had an average daily light intensity of 3433.34–15199.56 lux, demonstrated a slightly elevated NH_4^+ removal efficiency and duckweed productivity of 69.34% and 0.050 kg/m²/day, respectively. However, these values were not statistically significant compared to conditions without shade nets, with a removal efficiency of 63.97% and duckweed productivity of 0.042kg/m^2/day ($P<0.05$). Implementing shade structures that effectively decrease solar exposure by 25% shows promise for enhancing duckweed productivity and optimizing nutrient reduction in wastewater from fish cultivation systems. This approach contributes to the promotion of sustainable integrated aquaculture.

Keywords: duckweed, ammonium removal, aquaculture wastewater, light intensity, shade net

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1. Introduction

The global demand for fishery products, including aquaculture, continues to increase. It is estimated that world per capita fish consumption will reach 21.2 kg in 2030, representing an average increase of 20.5 kg since 2018–2020 (FAO, 2021). In Indonesia, fish consumption per capita was 54.5 kg in 2019 and increased by 3.47% to 56.39 kg per capita in 2020 (Harianto *et al.*, 2021), with the current level of fisheries cultivation production reaching 15.5 million tons in 2023 (KKP, 2024). This growth will continue and accelerate over the next decade (Obiero *et al.,* 2019). The government and industry are pursuing further intensification of aquaculture to meet the growing demand for fishery products. The intensification of aquaculture activities will have an impact on the increased use of inputs, primarily feed and water per unit area of land, as well as implications for the increased concentration of wastewater produced from the production system (Henriksson *et al*., 2018; Dauda *et al*., 2019). The nutrient content of aquaculture waste presents a potential increase in the pollution burden if discharged directly into the aquatic environment. The contribution of aquaculture activities to ocean and coastal waste nutrient inputs has increased sixfold worldwide, from 0.43 x 10^9 kg N/year in 1985 to 2.60 x 10^9 kg N/year in 2005 (Malone & Newton, 2020). The absence of waste management strategies can potentially negatively impact water quality and the equilibrium of aquatic ecosystems. This can result in the onset of eutrophication, a reduction in oxygen levels, and a decline in biodiversity.

In aquaculture waste management, the nutrient content of waste can be utilized and reused through the bioconversion process, whereby plants are employed to transform the waste into a form that can be used again. The utilization of duckweed in wastewater treatment techniques has been observed to reduce nutrient levels effectively. The protein content of duckweed biomass makes it a significant source of bioenergy and organic feed for fish farming operations (de Matos *et al*., 2014; Popa *et al*., 2017). In integrated aquaculture, using duckweed as feed can reduce cultivation costs and provide a remediation impact that reduces pollutant levels in wastewater, thereby reducing water pollution. Studies indicate that duckweed can save up to 85% on water conservation and up to 40% on feed expenditures (Chrismadha *et al*., 2019; Chrismadha, 2021; Paolacci *et al*., 2022).

Nevertheless, the cultivation of duckweed frequently encounters obstacles that impede its optimal growth, diminish production, and lead to inadequate nutrient absorption. Consequently, agricultural wastewater continues to exhibit elevated levels of nutrient concentrations. The substantial release of this wastewater into water bodies has the potential to induce pollution and eutrophication. Examining the environmental consequences associated with intensifying fishing output is important since it can result in heightened inputs, such as feed, and higher concentrations of wastewater.

The efficacy of duckweed in wastewater treatment and its integration into integrated aquaculture depends on the scale of growth or productivity exhibited by the duckweed. Two primary factors that impact productivity are nutrition and light intensity. NH_4^+ is a readily absorbable nutrient in cultivation wastewater, essential for duckweed growth. However, specific amounts of NH_4^+ have been found to exhibit toxicity (Tian *et al*., 2021; O'Mahoney *et al*., 2022). Furthermore, duckweed productivity is also influenced by light intensity. Both excessive and insufficient sunlight can negatively impact duckweed growth (Walsh *et al*., 2021; Megahud and Dalumpines, 2021). Furthermore, the rate of waste nutrient removal is contingent upon the productivity value of duckweed.

The concentration of NH_4^+ in aquaculture effluent strongly correlates with several factors of the cultivation cycle, such as fish age, feed quantity, and density. Currently, there is a lack of accessible data on duckweed's growth response and phytoremediation capacity when utilizing wastewater generated during a fish cultivation production cycle. This lack of information is particularly relevant to the impact of variations in ammonium concentration and fluctuations in natural sunlight intensity. The main objective of this study was to assess and improve the response and phytoremediation capacity of duckweed using aquaculture wastewater. By effectively incorporating duckweed into wastewater management and integrated aquaculture, the aim is to achieve sustainable implementation and generate additional value. This approach aligns with the government's promotion of the blue economy concept (Yadav *et al*., 2023; Bappenas, 2023).

2. Materials and Methods

The experiment was conducted in a greenhouse at the Limnology and Water Resources Research Center, National Research and Innovation Agency (RCLWR-BRIN), and observed for 18 days. The use of a greenhouse is the initial stage of this research to minimize limiting factors such as rainfall and pests. *Lemna perpusilla* species of duckweed, collected from the culture pond at RCLWR-BRIN, was employed in this study. Shade nets with 25 percent and 50 percent sunlight blocking were used as shade to ascertain the extent of reduction. A total of 36 plastic containers with dimensions of 0.61 m x 0.43 m x 0.38 m were utilized to cultivate duckweed. The experimental setup comprised a recirculation system with tanks measuring 2.0 m x 1.0 m x 0.5 m, a digital scale, and a rake for collecting duckweed. A randomized block design with three replications was employed to assess the influence of variations in catfish wastewater concentration on duckweed. A randomized block design was selected to regulate environmental variability within the greenhouse, thereby ensuring the accuracy of the assessment of the impact of light intensity on duckweed growth. Furthermore, the objective was to ascertain the response results obtained from the two sides of the factorial treatment and its interaction. The wastewater was classified into three categories based on the age of the fish (L1: 2 months, L2: 3 months, and L3: 4 months) and subjected to shade net treatment at 25% shade (N25), 50% shade (N50), and no shade (N0). The ammonium concentration test is carried out based on the APHA AWWA 4500-NH3-F-phenate method (2017) with a UV-Vis Spectrophotometer. Water quality in the cultivation media (pH and temperature) was measured using the HORIBA U-50 series multi-parameter water quality checker and a lux meter logger (Lutron LX-1128SD) installed in the greenhouse for 24 hours.

2.1 Configuration Design of Experiment

Duckweed cultivation containers are positioned within the recirculating system. The recirculation system is arranged using fiber tubs measuring 2x1x0.5 m3, arranged in tiers, and forms a recirculating water flow assisted by pumps and water towers (Figure 1a). The recirculation system is designed to regulate environmental conditions to ensure minimal variation, particularly in temperature, across different treatments. According to the treatment, each cultivation container is filled with media such as catfish cultivation waste (Figure 1b). Each container was filled with 50 liters of wastewater generated from catfish farming. This wastewater, categorized according to the age of the fish, contained different levels of ammonium, as shown in Table 1. Subsequently, 50 grams of duckweed were planted in each container. An 18-day observation period was conducted, during which samples were collected every three days to analyze the quality of duckweed and water.

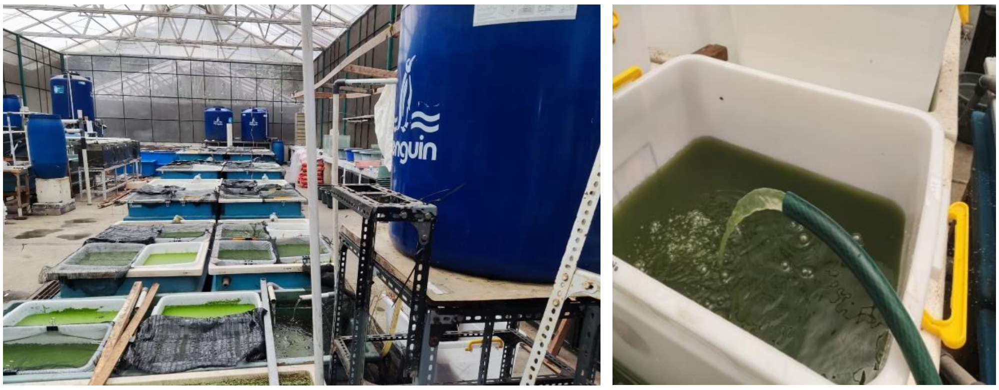

Figure 1. The installation of the recirculation system in experiment (a) and the filling of media/wastewater in (b).

Table 1. The ammonium concentration of wastewater sources

Wastewater Code	Fish Age in catfish farming	Ammonium (mg/L)
L1	± 2 months	3.57
L2	± 3 months	19.27
L3	± 4 months	35.64

During cultivation, a shade net was used to differentiate variations in sunlight intensity. The shading net used is a commercially available shading net with a 25% and 50% reduction range. The shade net is first calibrated to ensure the value of the percentage reduction range. Calibration was performed indoors by placing the lux meter under a commercial LED lamp at several measurement distances. Based on the measurement results, the light reduction value at N25 is 25.3±1.0%, and the 50% (N50) average reduction range is 49.8±2.2%. In this research treatment, there were two control groups (N0) without shade and (N0TL) without shade and duckweed. A lux meter logger was installed in the greenhouse for 24 hours to measure sunlight intensity at the research site.

2.2 Data analysis

Duckweed productivity is calculated referring to the following formula (Chrismadha *et al*. 2016):

$$P = \frac{W_t - W_0}{t.\ A} \qquad ...(1)$$

where: P = productivity, W_t = biomass at the time t, W_0 = initial of biomass, t = time dan A = surface area of the pond or container.

The removal efficiency of ammonium was calculated following the formula of Wang dan Sample (2013):

$$RE = \frac{C_0 - C}{C_0} \times 100 \qquad ...(2)$$

where: RE = removal efficiency (%), C = final concentration of pollutant (mg/L), dan C_0 = initial concentration the pollutant (mg/L).

3. Result and Discussion

3.1. The Light Intensity

According to the measurement results, the light intensity values in the greenhouse range from 0 to 50,100 lux. The light intensity value in the greenhouse is the same as the light intensity in the N0 treatment, with a daily average ranging from 3,433 to 15,199 lux (Table 2). Figure 3 shows the fluctuation of daily average light intensity values in each shade net treatment. These values are calculated as the average of the logging data per minute each day and will be used as data related to the correlation between parameters.

Table 2. Light Intensity in different shade nets during observation

Shade Net	Light intensity (lux)	Daily Average (lux)
N0	0- 50,100	3,433.34 – 15,199.56
N25	0- 37,575	2,575.01 – 11,399.67
N50	0- 25,050	1,716.67 – 7,599.78

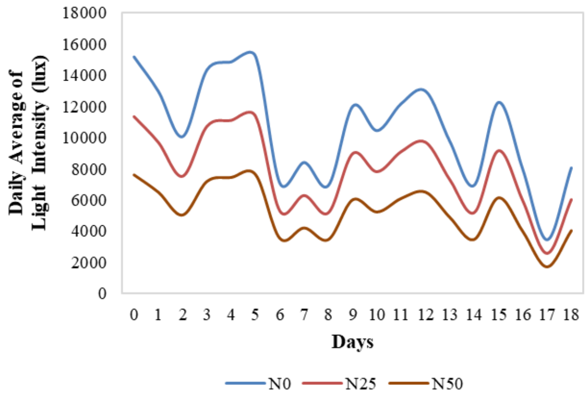

Figure 3. Light intensity fluctuation on shade nets

The intensity of outdoor sunlight varies depending on the weather, geographic location, and elevation, with illumination levels ranging from 130,000 lux on a sunny day to 15,000 lux in shady or cloudy conditions (Lanca *et al*. 2019). The fluctuation value outdoors is still higher than in the greenhouse, which served as the research location.

3.2 Duckweed productivity

The productivity of duckweed is inextricably linked to its growth rate, which is contingent upon the efficacy of photosynthesis. Peeters *et al.* (2013) stated that there is a linear correlation between photosynthesis and light intensity. In photosynthesis, plants need nutrients for optimal growth; plants' leaves and roots are required to capture light, water, and nutrients (Evans, 2013; Romand, 2024). Nutrient concentration and light intensity can have an interactive effect on duckweed growth. The application of shade nets to modify light intensity exposure, when integrated with disparate nutrient concentrations, elicits a dynamic response in duckweed productivity, as evidenced in Figure 4. The use of N0 and N25 when given low and medium nutrient concentrations (L1 and L2), productivity still gave a good response, compared to when at the highest nutrient concentration (L3), optimal growth only occurred on the third and sixth days then significantly decreased until the end of the study. In contrast, when employing the N50 method, the combination with L1 and L2 still exhibited productivity values, albeit lower than those observed with N0 and N25. Furthermore, when combined with L3, duckweed productivity was notably the lowest since the third day. In general, the N0 and N25 values, when combined with the three media (L1, L2, and L3), have higher average productivity values than the use of N50. Furthermore, there is no significant difference between the two. Nevertheless, there is a discernible tendency for N25 to yield slightly higher values than N0. The final results demonstrated that the productivity at N25 was 0.050 kg/m²/day, which was observed to be greater than the productivity at N50, which was recorded at 0.016 kg/m²/day. However, the productivity at N25 was similar to that without shade (N0), which was 0.042 kg/m²/day.

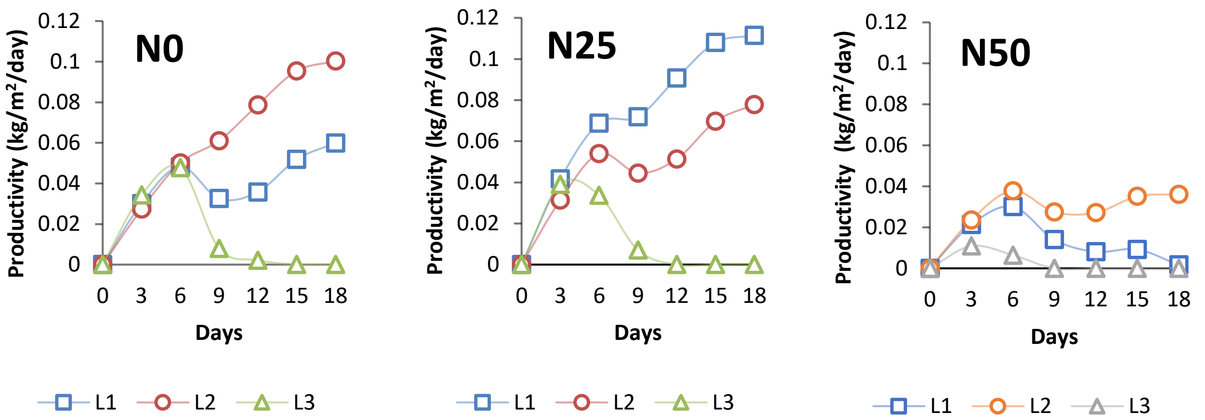

Figure 4. The duckweed productivity based on different shade nets on type media treatment

The productivity values declined when subjected to daily light intensity values of N50 compared to N25 and N0, even though duckweed was given media with small and medium concentrations (L1 and L2). The maximum light intensity value obtained by N50 was 25,050, with a daily average of 1,716.67–7,999.78 lux. This value remains within the range of optimal photosynthesis values. However, it is postulated that using N50 results in a lower frequency of optimal light exposure when compared to N0 and N50. The light intensity range encompasses the minimum threshold necessary to initiate photosynthesis, with the saturation point ranging from 300 μmol/ m^2.s (16,216 lux) to 600 μmol/m^2s (32,432 lux) at a temperature of 30°C. Oxygen evolution takes place within the light intensity range. Under photoinhibition conditions, duckweed's photosynthesis commences at a light intensity of 1,200 ¹mol/m^2s (64,864.86 lux). According to Landolt *et al.* (1987) and Wedge and Burris (1982), the ideal temperature for oxygen production in duckweed photosynthesis is 30°C, but the temperature range for CO_2 fixation is between 20 – 30°C.

The greenhouse, designated a research location of equivalent value to N0, exhibited a maximum light intensity of 50,100 lux (with a daily average of 3,433.34–15,199.56 lux). This value exceeded the optimal threshold but remained below the duckweed photoinhibition value. Conversely, N25 has the highest value, 37,575 lux (daily average 2,575.01 - 11,399.67 lux), within the optimal range. Therefore, N25 treatment can enhance duckweed photosynthesis, increasing growth and phytoremediation capacity. Good nutrient absorption, assimilation, transportation, photosynthesis, respiration, and enzymatic activity all play roles in the growth rate of duckweed (Landolt *et al.*, 1987). Photoinhibition occurs at a light intensity of

64,864 lux (Petersen *et al.* 2022), but light-induced stress in duckweed begins to appear at a light intensity of 54054.05 lux (Adams *et al.* 2020; Stewart *et al.* 2020). Despite being conducted in a partially enclosed environment (greenhouse) with a shade net as a treatment, the productivity value remained consistent with the previously reported findings. In a previous study, duckweed was cultivated in an integrated common carp (Cyprinus carpio) system with close recirculation aquaculture in an open area, resulting in a productivity range of 0.028 - 0.053 kg/m^2/day (Chrismadha *et al.*, 2016).

Based on the relationship between daily light intensity and productivity shown in Figure 5, the R^2 value of N0 and N25, when given media L1 and L2, duckweed productivity is stronger than when given media L3. While the R2 value at N50 when given low media concentration (L1) and high (L3), the value is smaller when compared to when given medium media L2; this indicates that when the light intensity is not optimal for growth, the presence of nutrients at the maximum growth points up to 18 days of maintenance, duckweed can still grow and survive. Megahud and Dalumpines (2021) reported that the highest growth or maximum growth point was achieved at a particular nutrient concentration and light intensity. Growth can continue to decline when nutrient concentrations decrease or successively increase beyond the maximum growth point, and growth will decline when light intensity is decreased or increased from the maximum growth point. Statistically, it was shown that the highest productivity value was shown in the use of N0 and N25 combined with L1 and L2 media, compared to when cultured in L3 media. Meanwhile, using L3 media in almost all combinations obtained the lowest value, especially when combined with N50 (Table 3.).

Table 3. The final productivity of duckweed

Treatment	N0	N25	N50
L1	0,043^{abcd}	0.083^{d}	0.013^{ab}
L2	0,070^{cd}	0,053^{bcd}	0,033^{abc}
L3	0,013^{ab}	0,013^{ab}	0,003^{a}
Average	0.042	0.050	0.016

Note: Different letters in the final productivity data indicate differences using the Duncan multiple range test ($P<0.05$)

The low productivity value is shown when using L3 media in all combinations with shade net. Specifically, L3 is a 4-month-old catfish wastewater with the highest initial NH_4^+ concentration of 35.64 mg/L. Duckweed productivity does not necessarily increase with high NH_4^+ concentrations, even though NH_4^+ is the compound most easily absorbed by plants. *Lemna minor* is known to grow well at NH_4^+ concentrations ranging from 7 to 138 mg/L, indicating that duckweed can tolerate high levels of NH_4^+. However, optimal growth occurs at a concentration of 28 mg/L (Huang *et al.*, 2013; Wang *et al.*, 2014). Although the NH_4^+ concentration in L3 is within the tolerance range, it exceeds the optimal value. As a result, duckweed survived until the end of the study but exhibited lower productivity (Figure 6). Optimal light intensity positively impacts productivity only if the media conditions support optimal growth. Which, in this case, is influenced by the ammonium concentration.

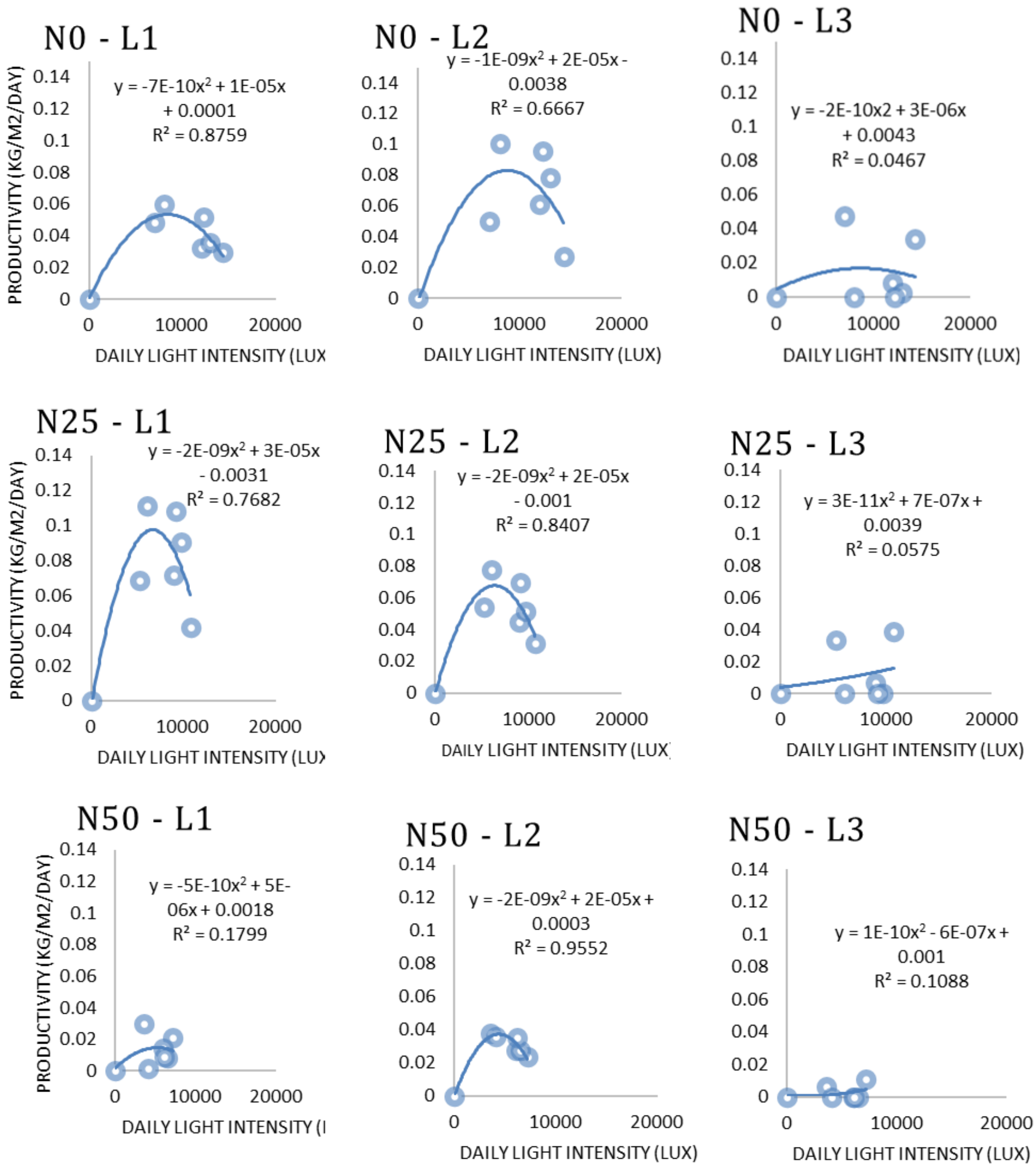

Figure 5. The daily light intensity-productivity relationship

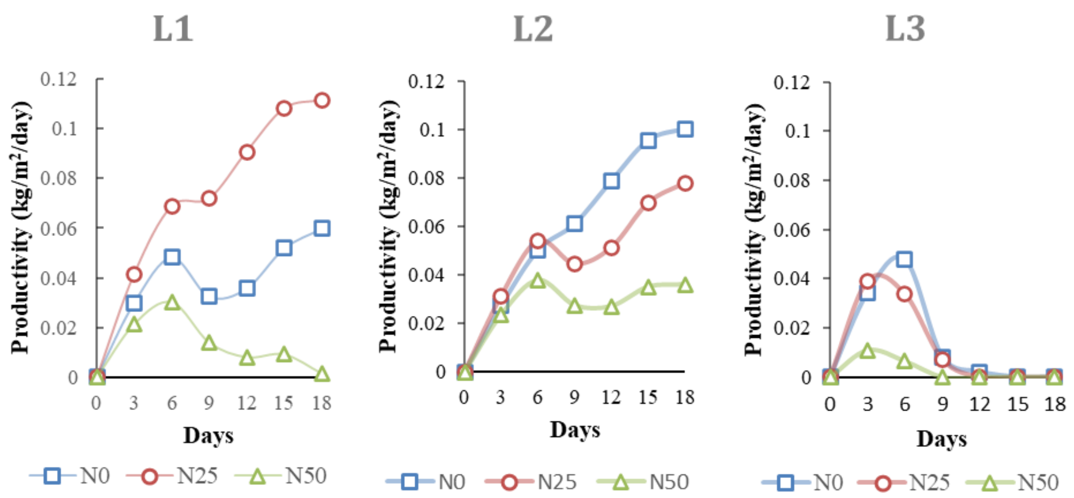

Figure 6. The duckweed productivity based on type media with shade net treatment

Environmental factors such as temperature and pH also influence duckweed productivity. Temperature can be influenced by light intensity; the higher the light intensity, the more the media temperature will increase. In this study, the media temperature was controlled by utilizing a recirculating system in duckweed culture, which can maintain the temperature within a range that did not vary significantly. Similarly, the pH levels in this study were within a range of values that were not significantly different except for the control, as seen in Table 4. The average media temperature and pH values remained within optimal ranges, namely 25 – 30 ^{0}C for temperature (Vymazal, 2008) and pH 5.0 – 7.5 (Mkandawire and Dudel, 2005; Vymazal, 2008). In the control treatment, the absence of duckweed caused higher pH and temperature values.

Table 4. Average temperature and pH of the media based on the shade net

Shade net	Temperature (^{0}C)	pH
N0	27.38 ± 0.2^a	7.78 ± 0.18^a
N25	27.41 ± 0.2^a	7.78 ± 0.15^a
N50	27.24 ± 0.3^a	7.75 ± 0.19^a
N0TL	27.69 ± 0.3^b	8.18 ± 0.21^b

Note: Different letters in the same column indicate differences using the Duncan multiple range test (P<0.05)

Table 5. The average removal efficiency of NH_4^+ in the duckweed culture media with the provision of shade nets

Treatment	N0	N25	N50	N0TL
L1	67,25	62,15	26,65	7,10
L2	74,35	83,90	72,35	36,80
L3	50,30	61,90	34,25	57,85
Average	63,97^b	69,32^b	44,42^a	33,92^a

3.3. Removal Efficiency Ammonium (NH_4^+)

During the 18-day observation period, the concentration of ammonium (NH_4^+) decreased in shade and unshaded media, indicating increased NH_4^+ removal efficiency by duckweed. The removal efficiency value fluctuated until the end of the research (Figure 7). The results show that N25 had a higher average removal efficiency (69.32%) compared to N50 (44.42%) and N0TL (33.92%). However, there was no significant difference between N25 and N0 (63.97%) (Table 5).

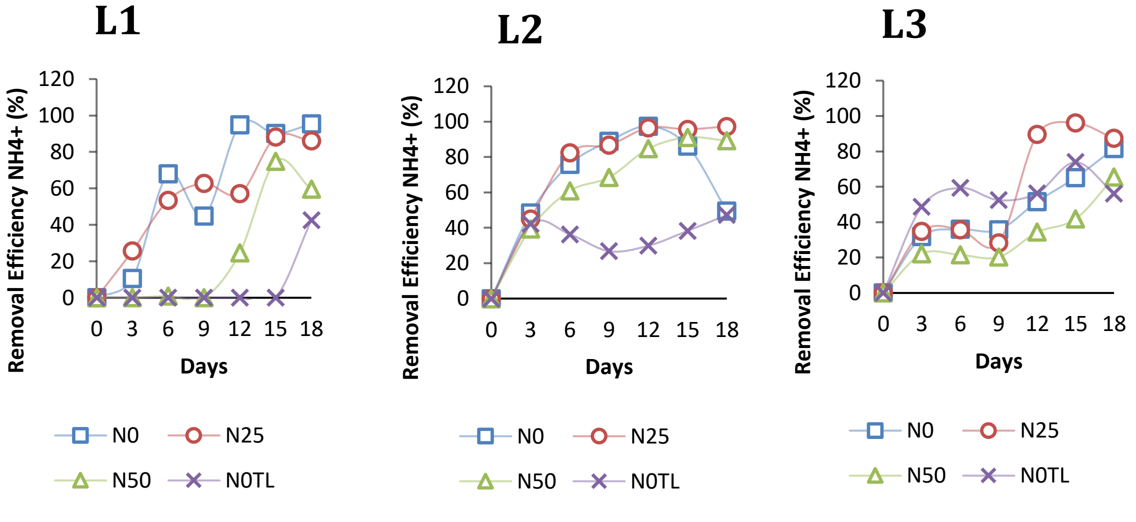

Figure 7. Fluctuation NH_4^+ removal efficiency on type media with shade net treatment

Daily light intensity is generally associated with duckweed growth and higher protein production (Femeena *et al.*, 2023). Duckweed growth is also associated with the reduction or uptake of nutrients in the media. The results of the experiment demonstrate that the use of N0 and N25 shade net on low (L1) and medium (L2) concentration waste resulted in higher removal efficiency values than when using high-concentration waste (L3). This finding aligns with the duckweed productivity value observed in Figure 6. The low productivity observed in L3 also resulted in a reduction in efficiency value that was low, even several times lower than that observed for N0TL. The addition of nutrients to L3 from duckweed that died due to high ammonium concentrations caused the rate of reduction efficiency to be slower in all three shade treatments.

Based on experimental results, high ammonium reduction, such as N25 and N0, is associated with the highest duckweed productivity, while low productivity results in low ammonium reduction values, such as N50. In nature, the Lemnaceae group grows in sunny and shady habitats, but shady habitats are preferred because of the lower light intensity and less extreme temperatures (Landolt, 1986). Plant reactions to different light intensities are also influenced by other abiotic factors such as temperature and nutrition (Francis and Gilman, 2019) and are also influenced by species (Petersen *et al.*, 2022). Several studies report that the light saturation of *Lemna minor* ranges from 342 to 400 μmol/m^2/s (18,486.49 – 21,621.62 lux) (Petersen *et al.*, 2022). Lack or excess of light intensity will have an impact on biomass growth, frond size, leaf pigments, root length, protein and starch content, as well as plant hormonal production (Femeena *et al.*, 2023; Strzalek and Kufel, 2021; Brini *et al.*, 2022). According to studies on *Lemna gibba*, light intensity higher than 1,000 μmol/m^2/s (54,054.05 lux) causes increased levels of zeaxanthin, which is a chlorophyll /photoprotection hormone against damage caused by intense light, detoxification of oxidants (reactive oxygen species/ROS, and other free radicals), and overall structural and functional maintenance of plant biological membranes. Zeaxanthin is also an essential micronutrient for humans if duckweed is developed for food (Adams *et al.*, 2020; Stewart et al., 2020; Petersen *et al.*, 2022). Another impact of very high light intensity exceeding the maximum limit for plants is that the rate of photosynthesis will decrease and can result in plant damage due to oxygen stress (photoinhibition) (Petersen *et al.*, 2022).

The effect of variations in daily light intensity through providing shade on NH_4^+ reduction efficiency can be seen directly in the correlation value. At N25, a firm determination coefficient (R^2) of 0.7598 and 0.8203 was obtained when combined with L1 and L2, compared to when combined with L3; this indicates that the optimal light intensity value will not produce good reduction efficiency if the optimal NH_4^+ concentration does not influence it. While the use of N0 indicates approaching the N25 value for the strength of the relationship, there is also an indication that it is equivalent to the use of N50 and NOTL. The light intensity produced by N0 indicates approaching a value close to the minimum limit of the stress level due to the influence of photoinhibition, which results in disrupted growth and reduced efficiency. Even though the values are not significantly different, this is a sign that increasing light intensity beyond N0 can cause suboptimal growth and improve the effectiveness of duckweed remediation in removing NH_4^+ (Figure 8).

Other factors besides light intensity that also influence NH_4^+ reduction efficiency in the use of aquatic macrophytes such as duckweed include the initial NH_4^+ concentration, macrophyte tolerance level, biomass, and media water quality such as temperature and pH (Kinidi and Salleh, 2017; Walsh *et al.* 2021; Fahmi *et al.*, 2023).

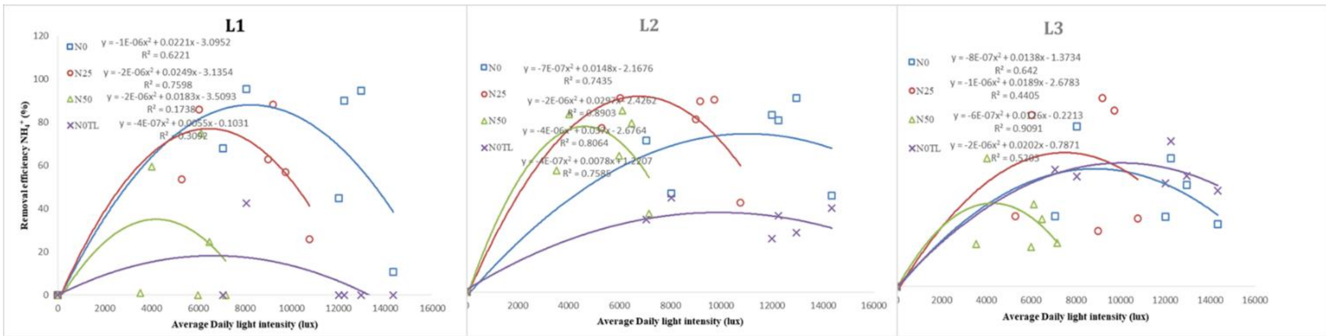

Figure 8. Relationship between daily average light intensity and NH_4^+ removal efficiency on different media with varying shade net

The initial concentration of NH_4^+ in the media is thought to determine the growth rate of duckweed biomass and its phytoremediation capacity. The initial concentrations of NH_4^+ in this study were L1 (3.57 mg/L), L2 (19.27 mg/L), and L3 (35.64 mg/L), which according to quality standards were in the high category (PP No. 22 of 2021). Despite being duckweed's most preferred compound, a high concentration of NH_4^+ does not guarantee good duckweed growth and good nutrient uptake. High levels of NH_4^+ can inhibit the absorption of cations such as calcium and magnesium from the substrate, causing a deficiency of these elements in plants. A decrease in the uptake of these essential cations can cause more problems for plant growth and metabolism (Zhao *et al*. 2016). As in the results of this study, in terms of media, the best average reduction efficiency value is owned by L2 with a value of 76.87% and is significantly different from L1 (52.02%) and L3 (48.82%) (Figure 7. Despite L3 having the highest NH_4^+ content, it exhibits lower duckweed uptake compared to L2 media, which is in line with the previous explanation that a high NH_4^+ concentration does not guarantee good duckweed growth and nutrient uptake, even though NH_4^+ is the compound most easily absorbed by plants. Meanwhile, in L1, as the medium with the lowest initial concentration of NH_4^+, a high NH_4^+ reduction efficiency value should be obtained, but the results show that the value tends to be low; it is suspected that there is an influence of shade in the process, where the shade of 50% (N50) when using L1 media has the lowest reduction efficiency level.

NH_4^+ is a nitrogen compound most easily absorbed by plants and nitrate (NO_3^-). However, ammonia can decrease duckweed productivity at high concentrations because it can be toxic, causing root and leaf rot and inhibiting nutrient uptake in the media. Symptoms of NH_4^+ poisoning in plants include reduced growth, leaf chlorosis, changes in root shape, decreased root/shoot ratio, decreased root gravitropism, and triggering oxidative stress (Caicedo *et al*., 2000; Liu and Wiren, 2017; Tian *et al*., 2021). Oxidative stress can be caused by oxygen deficiency, including direct photoreduction of O_2 to O_2^- via reduced electron transport associated with the photo-respiration cycle (Wang *et al*., 2014). Excessive increases in reactive oxygen species (ROS) due to oxidative stress can also result in oxidative damage to proteins, DNA, and lipids. ROS production is one of the leading causes of reduced productivity, injury, and death accompanying these plant stresses (Mittler *et al*., 2004; Huang *et al*., 2013). Most plants will experience toxicity when NH_4^+ is in the millimolar (mM) concentration range or is the only nitrogen source, and in the micromolar range (lower than mM), most species roots prefer ammonium uptake over nitrate (Gazarrini *et al*., 1999; Britto and Kronzucker, 2002; Liu and Wiren, 2017).

According to Nasr *et al*. (2009), although duckweed is susceptible to NH_4^+ concentrations, it can still treat wastewater containing very high concentrations of total ammonia if a certain pH level is not exceeded. Through additional oxygen supply, degradation of organic matter can be increased by duckweed and additional area surface for the growth of bacteria and algae, which can contribute to the total loss of nutrients in shallow systems, regardless of loading rates (Korner *et al.*, 2003).

Ammonium (NH_4^+) and nitrate (NO_3^-) are the primary forms of inorganic nitrogen in wastewater that plants can absorb directly. The energy required for the assimilation of NH_4^+ is lower than that required for the assimilation of NO_3^-. NH_4^+ is the preferred nitrogen source for many plants in the micromolar (μM) concentration range (Tian *et al.* 2021). Unlike other plants, duckweed has better ammonium absorption than other nitrogen sources (Porath and Pollock, 1982). However, at high concentrations, ammonium ions inhibit duckweed growth. Growth inhibition by total ammonia (NH_4^+ + NH_3) is generally caused more by the NH_3 form than by the NH_4^+ form (Caicedo *et al.*, 2000).

Duckweed biomass also has a crucial role in the level of NH_4^+ reduction efficiency. The amount of biomass, density, and surface area of duckweed cover significantly influence the level of reduction efficiency. High duckweed density can negatively impact growth and has implications for developing duckweed-based remediation systems (Paolacci *et al.*, 2022). High density can result in some individuals lacking sufficient sunlight, not being adequately exposed to the media, or being submerged up to the leaves, hindering their photosynthesis ability. Duckweed density also affects oxygen levels in the medium (Walsh *et al.*, 2021). The influence of light intensity on duckweed productivity has been demonstrated to be significant. To understand how duckweed productivity influences NH_4^+ reduction efficiency, the correlation between productivity and NH_4^+ reduction efficiency is depicted in Figure 9. At N25, there is a slightly stronger correlation with no shade (N0) than with duckweed at N50, indicating that optimal light conditions (N25) enhance both productivity and NH_4^+ reduction efficiency.

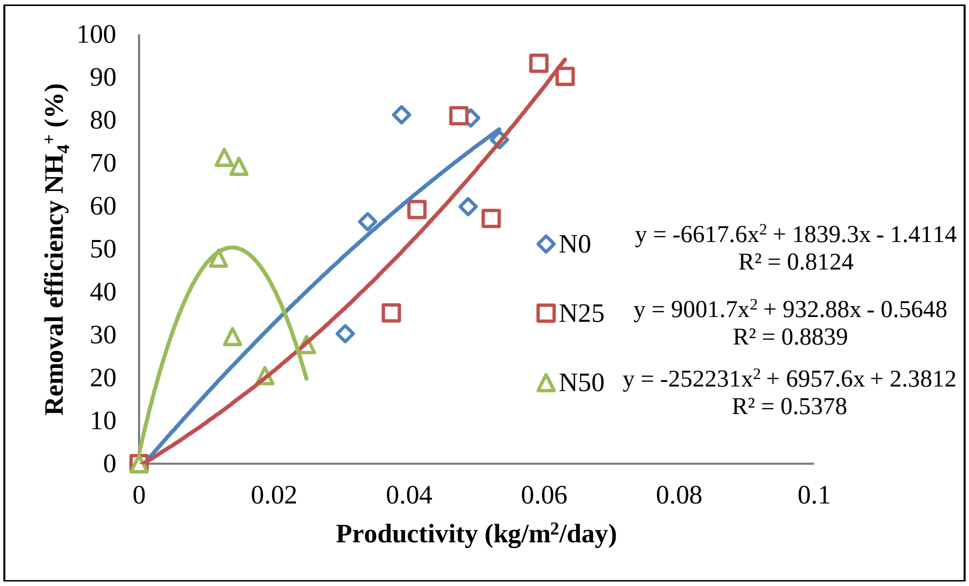

Figure 9. Duckweed productivity-removal efficiency NH_4^+ relationship based on shade net variations

The response of duckweed to ammonium (NH_4^+) and ammonia (NH_3) levels is widely reported in the literature, but the conclusions do not always agree. Shen *et al.* (2019) reported that the NH_4^+ removal efficiency from the combination of *Acinetobacter* sp. and duckweed strains in media and cultivation wastewater samples at a temperature of 15°C exceeded 99%. Ahmadi and Dursun (2024) reported that the removal efficiency of NH_4^+ from the secondary clarifier tank of a conventional biological treatment system after the settling process reached 72%. Sarkheil and Safari (2020) explained that the use of *Lemna minor* in phytoremediation in the cultivation of African cichlid fish (*Labidochromis lividus*) was able to reduce total ammonia nitrogen (TAN) by 43.7% after 48 hours and seven days. It isn't easy to compare the results of the studies mentioned above because they were obtained under different conditions of temperature, pH, wastewater type, and media composition.

The differences in NH_4^+ removal efficiency revealed in this study are consistent with previous studies, emphasizing the complex relationship between duckweed productivity, light intensity, and NH_4^+ concentration. The correlation between duckweed productivity and NH_4^+ removal efficiency, shown in Figure 9, emphasizes the significance of managing environmental conditions to improve phytoremediation outcomes. Specifically, delivering optimal light intensity (observed with N25) appears to support higher productivity and improved NH_4^+ reduction efficiency.

4. Conclusion

The media's light intensity and nutrient concentration are critical factors for duckweed growth. These factors can independently or collaboratively influence growth outcomes. The optimal conditions for light intensity and ammonium concentration yield better growth effects. In contrast, suboptimal conditions or conditions that exceed the optimal value limit can lead to reduced productivity, induce toxic effects, and impede the efficacy of duckweed-based remediation. The use of shade nets that reduce sunlight by 25% (N25), which produces an average daily intensity of 2575.01 - 11399.67 lux, encourages slightly higher growth than duckweed without shade (N0), with an ammonium concentration of 19.57 mg/L still obtained good duckweed productivity, while at a concentration of 35.64 mg/L obtained low productivity. It is anticipated that the findings of this study will serve as a point of reference for developing strategies for utilizing duckweed in waste management, water quality control, and biomass production in integrated aquaculture systems. The results of this research still need to be tested outdoors with environmental factors such as weather, land/pond conditions, pests, and other factors that may have different effects. Future research could focus on refining the balance between nutrient levels and light conditions to maximize duckweed's utility in various environmental and aquaculture settings. Overall, this study provides valuable insights into optimizing conditions for duckweed in aquaculture, offering a promising approach for enhancing wastewater treatment and supporting sustainable fish farming practices.

Data availability statement

The data included and used in this study is not confidential and is available upon request.

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Conflict of interests

All authors have stated that there is no conflict of interest in relation to the manuscript's writing or submission.

Authors contribution

AW, **KN**, **AS**, **YPH**, **TC** and **ES** as the main contributors, conceptualized the study, data analysis, writing-review, editing and funding acquisition. **FSL**, **EN**, **SG**, and **NM** support laboratory analysis, data collection, and data analysis.

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