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Investigation of Nutrient Removal Capacity and Growth Rate of Duckweed (*Lemna minor*) Under Different Harvesting Protocols in Aquaponics

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Abstract

In aquaculture systems, a high proportion of nutrients end up in the water as a by-product of metabolic processes. These must be neutralized through filtration, but to increase efficiency, the integration of some aquatic plants is advisable. Through the nutrient uptake capacity of these plants, the environmental impact of aquaculture systems can be decreased, so they become more sustainable. In this experiment, common duckweed (*Lemna minor*) was used under different harvesting protocols (control, and 25% and 50% of surface area harvested) to examine the nutrient uptake capacity of the plant and the effects on fish (common carp—*Cyprinus carpio*) production parameters. It can be concluded that the treatments used did not have a significant effect on fish production parameters. However regular duckweed harvesting had a positive effect on the plant's biomass production and daily growth rate. By the end of the experimental period, the harvested groups had accumulated more biomass than the control group, though there was no difference between the 25% and 50% harvest rates. In our experiment, the control group achieved a yield of 17.9 t/ha/year, while the regularly harvested (25% and 50%) treatments achieved yields of 23.4–24 t/ha/year (based on extrapolated data). Regular harvesting of duckweed resulted in lower ammonia levels, as the free water surface available to the plants after harvesting allowed for more intensive growth, enabling them to absorb more organic matter. The dynamics of nitrite, nitrate and orthophosphate concentrations are primarily determined by the internal biochemical processes of the system and temporal development, while treatments such as duckweed harvesting had no direct effect on these parameters.

Keywords: aquaculture; aquaponics; common duckweed; water quality; wastewater management; nutrient retention; nitrification; biological filtration; water recycling and reuse; monitoring

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Academic Editor: Reynaldo Patiño

Received: 16 October 2025
Revised: 3 November 2025
Accepted: 5 November 2025
Published: 6 November 2025

Citation: Molnár, P.I.; Bényi, B.C.; Bársony, P.; Posta, J.; Fehér, M. Investigation of Nutrient Removal Capacity and Growth Rate of Duckweed (*Lemna minor*) Under Different Harvesting Protocols in Aquaponics. *Water* **2025**, *17*, 0. https://doi.org/

1. Introduction

Aquaculture is the fastest growing food production sector in the world [1]. It plays an indisputably important role in human nutrition, food security, and food safety [2,3]. The common carp (*Cyprinus carpio*) is one of the most widely produced fish species in aquaculture in Central Europe. In 2022, Poland produced 18,000 tons, the Czech Republic 16,437 tons, and Hungary 11,057 tons of carp [4]. These three countries together account for 71.2% of the EU's carp production [5]. Based on these figures, it can be said that carp occupies an important place in aquaculture production in Europe and within Hungary. Its economic importance, good environmental tolerance [6], and high growth performance [7] make it an excellent candidate for use as a model animal in various experiments.

In aquaculture systems, approximately 60% of the nitrogen is introduced through feed; it is generally very high in protein, and is excreted into the water through the metabolic processes of fish [8]. Therefore, it is neutralized using physical and biological filters [9]. The key process in biological filtration is nitrification, in which nitrifying bacteria convert ammonia (NH_4) in the water into nitrates ($NO_3{}^-$) through a two-step oxidation process [10–13]. Since oxygen is required for oxidation reactions during this process, the activity of nitrifying bacteria is particularly intense in oxygen-enriched aquatic environments [14]. The role of nitrification is particularly important in aquaculture and wastewater management [15]. Wastewater management helps in reducing the environmental impact by converting ammonia found in wastewater and aquaculture effluent into harmless nitrates.

The integration of certain aquatic plants into aquaculture systems can reduce the environmental impact of aquaculture production, primarily due to their nutrient uptake capacity, thus increasing the efficiency of biological filtration and making it more sustainable [16]. Due to their many beneficial properties, duckweed (*Lemnaceae*) species may serve as a potential solution as wastewater treatment plants [17]. Plants belonging to the *Lemnaceae* family are among the smallest macrophytes in the world. They can be found almost everywhere in the world, in both still and flowing waters. These floating aquatic plants form mats on the water surface [18,19] and reproduce primarily vegetatively [20]. Duckweed species are considered among the most promising aquatic plants for wastewater treatment due to their potential [21,22], as they can absorb large amounts of nutrients [23]. According to some studies, duckweed species can recover 96–99% of ammonia compared to the initial concentration in 3 days [24], while others claim 83.7% of total nitrogen and 89.4% of total phosphorus (TP) can be removed by duckweed in 8 weeks [25]; furthermore, duckweed combined with some strains of bacteria can remove 99% ammonia in aquatic systems [26]. In aquatic systems, duckweed proved to be a great resource in enhancing the systems's biofilter unit [27]. Furthermore, they are effective in removing heavy metals and organic chemicals, and they also reduce biological oxygen demand and chemical oxygen demand [24,28–30]. Duckweed-based systems simultaneously purify wastewater and recover nutrients. This provides a cost-effective and sustainable solution to the problem of wastewater treatment [31]. At the same time, large amounts of protein-rich plant biomass can be produced in a short period of time, with a protein content of up to 41.74% on a dry matter basis [32], which can be used in both animal feed and human nutrition [33–35].

Reducing the environmental impact of intensive aquaculture systems is essential. The effluent from these systems is rich in organic matter, the release of which into the environment can cause serious problems. A solution to this problem may be provided by aquaponics production technology, which is becoming increasingly prominent today. This production technique combines aquaculture and hydroponic technologies, allowing us to take advantage of their benefits to reduce the environmental impact and increase the sustainability of aquaculture production [36].

Aquaponics systems are a form of integrated aquaculture systems that combine aquaculture and hydroponics [37], where a symbiotic relationship develops between fish, microbes, and plants [38]. The importance of these systems is becoming increasingly prominent as they reduce water consumption, increase production profitability, recycle nutrients, and reduce the environmental impact of the aquaculture sector.

There are three main strategies for crop production in aquaponics systems: staggered cropping, intercropping, and batch cropping [39,40]. In staggered cropping, different groups of plants are harvested at different times, which helps to maintain a constant nutrient uptake. However, excessive harvesting can lead to nutrient accumulation and plant death due to a reduction in plant biomass [41]. Although several studies have shown that harvesting can improve nutrient removal [42,43], other sources stated the opposite [44], so there is no consensus on this subject [45].

In our previous studies using *Spirodela polyrhiza*, the following harvesting protocols were investigated: 0%, 25%, 50%, 75%, and found that the 75% harvesting ratio was counterproductive, meaning that the duckweed could not replenish itself quickly enough for such a high rate of harvests weekly [46]. A similar study investigated watercress (*Nasturtium officinale*) with harvesting rates of 0%, 25%, 33%, 50% [45]; watercress showed increased biomass production in the 25% treatment, but the increase in harvested biomass had negative or no effects at all on nutrient removal efficiency.

The aim of present study was to investigate the effects of different common duckweed harvesting protocols on aquaponic systems. In this context, we examined the production indicators of duckweed (growth rate, yield), the water quality parameters of the system (ammonia, nitrite, nitrate, orthophosphate concentrations, dissolved oxygen), and the production parameters of fish raised in the same system. This study thus provides information not only on the optimal harvesting strategy for duckweed, but also on its impact on the aquaponics system.

2. Materials and Methods

2.1. Test Environment

The experiment was conducted in the greenhouse of the Aquaculture Laboratory of the Faculty of Agricultural and Food Sciences and Environmental Management at the University of Debrecen. For the purposes of the study, we set up nine small aquaponics systems, each with a water volume of 200 L (150 L for fish farming and 50 L for plant cultivation). Each system consisted of three units: a fish farming unit, a plant cultivation unit, and a filter unit (Figure 1). The hydroponic (plant cultivation) unit provided 0.4 m^2 of surface area for duckweed. The systems included a pump (Tetra WP 600, Tetra GmbH, Melle, Germany), a heater (Tetra HT 150 W, Tetra GmbH, Melle, Germany), a mechanical filter unit (filled with 10 L of LECA clay pellets), and aerator stones (A962) connected to an air compressor (150 L/minute). The plants were illuminated with 100-watt Optonica LED lamps (Guangzhou Optonica LED Co., Ltd. Zhongshang China) for 12 h a day, providing 35,000 lux of light intensity. The experiment lasted 56 days.

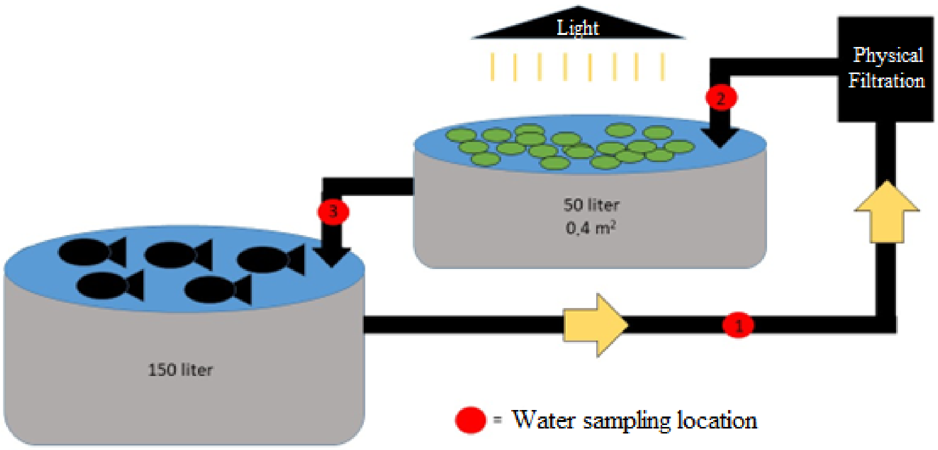

Figure 1. Schematic diagram of the aquaponics system and sampling protocol used in the experiment.

2.2. Experimental Stock

The common duckweed (*Lemna minor*) and common carp (*Cyprinus carpio*) used in the experiment came from the Aquaculture Laboratory's own stock. In total, 120 g of wet duckweed biomass and 10 carp juveniles were placed in each tank, with an average weight of 38.24 ± 0.67 g at the start of the experiment and a total biomass of 382.4 ± 6.73 g.

2.3. Treatments

Based on the harvesting protocols, three treatments were developed: control (0% removal), 25% (25% of surface area), and 50% (50% of surface area). The treatment groups were arranged in a block design. In the 25% and 50% groups, 25% and 50% of the duckweed produced on a weekly basis was harvested, a total of 8 times, while in the control treatment, no plant biomass was removed during the experiment. The amount removed was determined as a percentage of the surface area (in m^2). For this reason a plastic divider was made, to divide the tank into 4 equal parts, and we harvested 25 and 50%, respectively, in the treated groups. This divider was necessary to prevent the duckweed in the tank from floating into the harvested quarters.

2.4. Initial Water Composition and Replacement

The water used initially to fill up the systems and for water replacement had the following water quality parameters: ammonia: 0.09 ± 0.01 mg/L, nitrite: 0.005 ± 0.001 mg/L, nitrate 0.3 ± 0.0 mg/L, orthophosphate 0.004 ± 0.001 mg/L. During the experimental period, no water change was undertaken, and only the evaporated water was replaced. This means 5% water replacement on average.

2.5. Water Quality Measurement

The oxygen concentration (8.46 mg/L ± 0.16 mg/L) and temperature (19.57 °C ± 0.2 °C), as well as the pH (8.36 ± 0.15) of the water, were monitored daily (Hach Lange HQ30d, Hach Lange GmbH, Düsseldorf, Germany). We also measured the ambient temperature and light intensity daily (Peaktech 5065(PeakTech Prüf- und Messtechnik GmbH, Ahrensburg, Germany)—light intensity 6279 ± 2595 Lux; PCE THB 40 (PCE Deutschland GmbH, Meschede, Germany)—temperature 17.03 ± 4.10 °C and humidity 64.44 ± 8.79%).

On a weekly basis, prior to harvesting and on the day following harvesting, we measured the water's nitrogen (ammonia—NH_3-N—Method 8038, nitrite—NO_2-N—Method 8507, nitrate—NO_3-N Method 8171) and phosphorus (orthophosphate—PO_4-P—Phosphormolybdenum Blue method) in the water using a HACH Lange DR/3900 (Hach Lange GmbH, Düsseldorf, Germany) spectrophotometer. Water samples were taken from three locations in each system on each occasion. Before (1) and after (2) the physical filter and after the plant production unit (3) (432 samples in total).

2.6. Carp and Duckweed Production Parameters

The following fish production parameters were examined: survival rate (%), individual body weight (g), biomass (g), weight gain (g), Specific Growth Rate-fish (SGR, %/day), Feed Conversion Ratio (FCR, g/g), homogeneity (CV%). The following plant production parameters were examined: biomass (g), Specific Growth Rate-plant (SGRplant, %/day).

The following formulas were used to evaluate the results:

$$\text{Survival} = 100 \times (\text{nt}/\text{n0}) \tag{1}$$

$$\text{WG} = \text{Wt} - \text{W0} \tag{2}$$

$$\text{SGRFish}\ (\%/\text{day}) = 100 \times (\ln\text{Wt} - \ln\text{W0})/\text{days of experiment} \tag{3}$$

$$\text{FCR (g/g)} = \text{WF (g)}/\text{WG (g)} \tag{4}$$

$$\text{CV\%} = (\text{SD}/\text{M}) \times 100 \tag{5}$$

$$\text{SGRPlant (\%/day)} = 100 \times (\ln\text{FB} - \ln\text{IB})/\text{t} \tag{6}$$

Wt: final body weight;
W0: initial body weight;
CV%: homogeneity;
SD: standard deviation;
M: mean;
WF: Weight of feed (consumed);
WG: Weight gain;
FB: Final biomass;
IB: Initial biomass;

2.7. Feeding

The carp were fed using JBL autofood (JBL GmbH & Co. KG, Neuhofen/Pfalz, Germany) automatic feeders, 4 times a day, receiving 1% of their body weight each day (because of the relatively low water temperature). We fed the fish with commercial feed with a particle diameter of 1 mm (52% crude protein, 20% crude fat).

2.8. Sampling

During the experimental period, the fish were weighed on a weekly basis and the feed rations adjusted, and the duckweed biomass was weighed using a digital scale (VWR LP-6501 (Avantor Inc., Radnor, PA, USA), max: 6500 g, 0.1 g accuracy). The duckweed was harvested every Monday, and the removed wet plant biomass was centrifuged for 2 min and weighed.

2.9. Water Quality Sampling Protocol

During the experiment, water samples were taken weekly from the aquaponics units and the concentrations of ammonia NH_3–N, nitrite NO_2–N, nitrate NO_3–N, and orthophosphate PO_4–P were determined for all samples (HACH Lange DR/3900 spectrophotometer). The sampling protocol for all aquaponics units was as follows: before and after harvesting; before (1) and after (2) the physical filter, and after the plant production unit (3).

The data obtained were evaluated based on the following factors:

Effect of time: the effect of sampling times on water quality parameters. During the experiment, weekly water samples were taken from the units and the average concentrations of ammonia NH_3–N, nitrite NO_2–N, nitrate NO_3–N, and orthophosphate PO_4–P were measured, independently of the sampling location, harvest time, as well as the applied treatment (n = 54 samples per week).

Effect of sampling point: the effect of sampling points (1, 2, 3) on water quality parameters. During the experiment, the average concentrations of ammonia NH_3–N, nitrite NO_2–N, nitrate NO_3–N, and orthophosphate PO_4–P in water samples taken from three different points of the units, independently of the sampling date, harvest date, as well as the applied treatment (n = 162 samples per sampling point).

Before/After harvest: changes in water quality parameters before and after plant harvesting. During the experiment, the average concentrations of ammonia NH_3–N, nitrite NO_2–N, nitrate NO_3–N, and orthophosphate PO_4–P in water samples taken from the units before and after harvesting, independently of the sampling time, sampling location, as well as the applied treatment (n = 243 samples per measurement, including control).

Effect of treatment: the effect of the applied treatments on the water quality parameters. During the experiment, the average concentrations of ammonia NH_3–N, nitrite NO_2–N, nitrate NO_3–N, and orthophosphate PO_4–P in water samples taken from units affected by different treatments, independently of the time of sampling, the sampling location, and the time of harvest ($n = 162$ per harvesting protocol).

2.10. Statistical Analysis

All evaluated parameters were received from the block design experiment. The effect of treatment on the production parameters of fish (survival rate, individual body weight, biomass, weight gain, SGR, FCR, homogeneity) and plants (biomass, SGRplant) were evaluated using one-way analysis of variance. In case of significant difference ($p < 0.05$), Tukey's-test was used for pairwise comparison.

The water quality measurement results were statistically compared using multivariate analysis of variance. The effects of sampling location, harvest, time, and treatment were considered as fixed effects in the model. If the factor proved to be significant ($p < 0.05$), Tukey-test was used to perform pairwise comparisons within the factors. The statistical evaluation was performed using the SPSS 26 software package.

3. Results

3.1. Fish Production Parameters

Table 1 shows the production parameters of the fish. Based on the data, it can be stated that the duckweed harvesting protocols used had no effect on fish growth and other production indicators. The treatments did not affect survival rate, specific growth rate (SGR), feed conversion ratio (FCR), weight gain, or fish homogeneity (CV%). Thus, it can be stated that the integration of duckweed harvests can be a viable solution for practical use without affecting fish production in any way.

Table 1. Fish production parameters.

	Control	**25**	**50**
Average initial weight (g)	38.39 ± 0.64	37.97 ± 0.66	38.37 ± 0.67
Average final weight (g)	64.63 ± 13.75	63.31 ± 14.01	64.70 ± 11.44
Survival rate (%)	90.00 ± 10.00	96.67 ± 5.77	86.67 ± 15.27
SGR_{Fish} (%/day)	0.98 ± 0.032	0.90 ± 0.067	0.98 ± 0.072
FCR (g)	0.83 ± 0.13	0.89 ± 0.23	0.85 ± 0.22
Weight gain (g)	26.24 ± 4.02	25.35 ± 7.22	26.33 ± 6.69
Final biomass (g)	601.67 ± 13.20	594.00 ± 114.64	554.33 ± 48.81
CV%	21.36 ± 5.72	22.58 ± 6.59	17.81 ± 2.09

$p < 0.05$

3.2. Water Quality Parameters

3.2.1. Effect of the Factors Examined on the Ammonia Concentration in Water

All the factors examined influenced the ammonia concentration during the experimental period (Figure 2).

Time as a factor clearly influenced the amount of ammonia dissolved in the water, as we did not change the water in the systems during the experiment, and so we did not remove organic matter from it. However, we fed the fish a certain amount of feed every day, and the ammonia level in the water gradually increased due to the uneaten feed and the fish's metabolic products. There was no difference in the first two weeks, but in the third week, the ammonia level was higher than in the first week. The value continued to increase in the fourth week. The next significant difference appeared in the seventh week. There was a difference between the values in the eighth and ninth weeks, but neither differed from the result in the seventh week.

In addition to time, the sampling location also influenced the ammonia content of the water. The results of the samples taken at the physical filter show that the ammonia level was lower than the values of the samples taken at the duckweed unit or the fish farming unit.

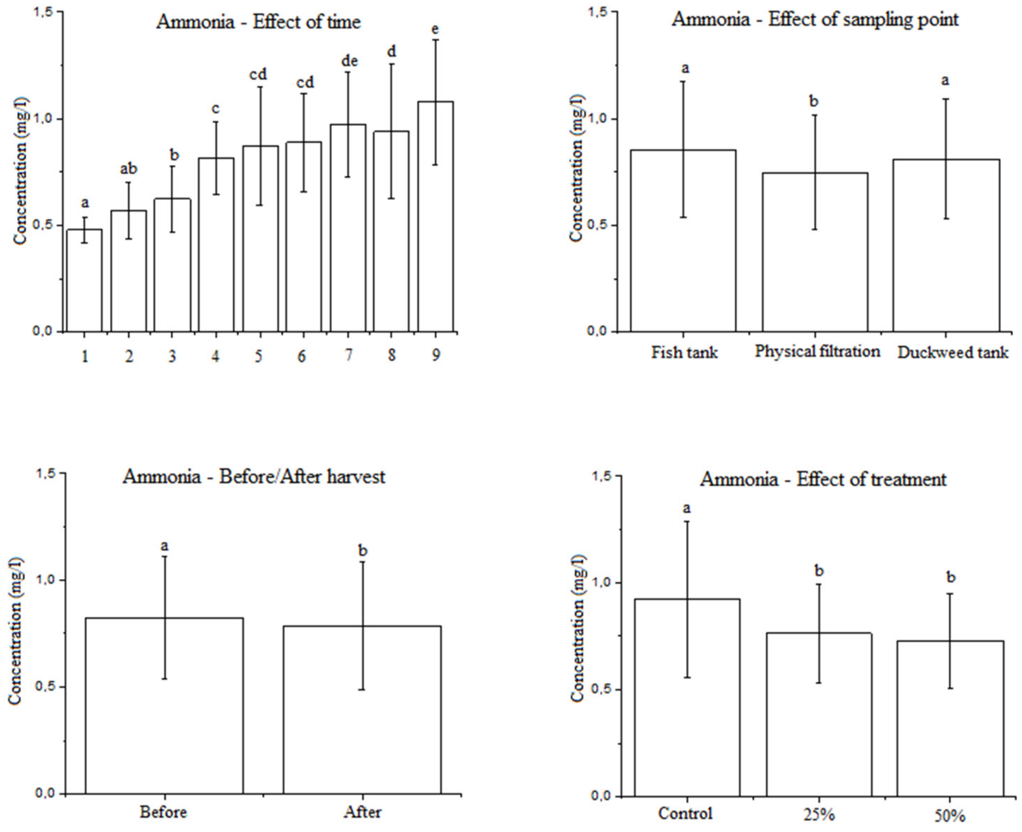

Figure 2. Effect of the factors studied on changes in ammonia concentration. The results are averages and standard deviations for each average. The values represent concentration. The different letters indicate significant differences ($p < 0.05$).

Harvesting also influenced ammonia levels. Lower values were measured in samples taken on the day after harvesting. After harvesting, there was free water surface for the duckweed, allowing it to produce new leaves, so the water surface did not hinder its growth, allowing it to absorb more organic matter from the water to support its growth rate.

The results of the treatments show that in units where regular harvesting took place, regardless of quantity, this influenced the amount of ammonia in the water. In these units, we measured lower levels compared to the control group. According to [47], duckweed prefers ammonia among nitrogen forms, which is confirmed by the difference in concentrations observed in this experiment between the treatments.

3.2.2. Effect of the Factors Studied on the Nitrite Concentration in Water

In the case of nitrite concentration (Figure 3), only the effect of time proved to be significant among the factors examined. The highest concentration was measured in the first week, it decreased in the second week, and the lowest nitrite level was reached in the third week. There was no difference between the measurements taken in the third, fourth, and fifth weeks. In the sixth week, the level rose to match the concentration measured in the second week, but there was no difference between the data from the sixth, seventh, and eighth weeks. The results for the seventh and eighth weeks were lower than those for the second week. The nitrite concentration measured at the end of the experiment was the same as the data measured in the third week, but also the same as those for the seventh and eighth weeks.

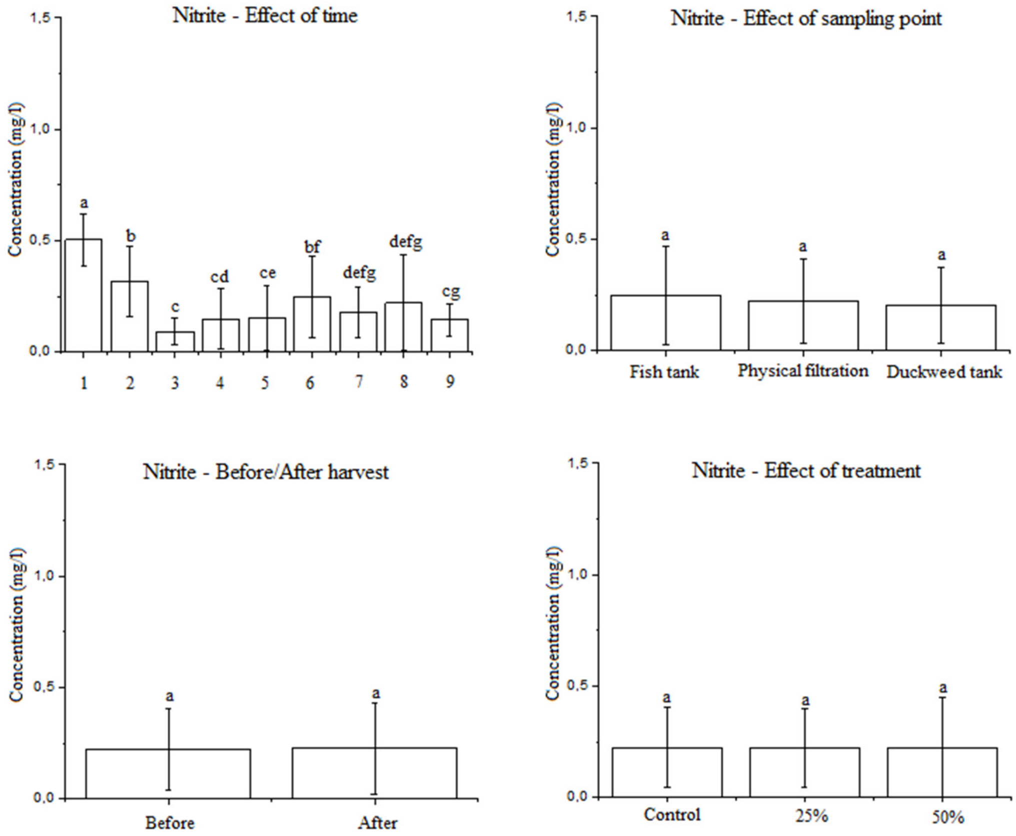

Figure 3. Effect of the factors studied on changes in nitrite concentration. The results are averages and standard deviations for each average. The values represent concentration. The different letters indicate significant differences ($p < 0.05$).

The results showed that sampling location and harvesting alone had no effect on the concentration of nitrite dissolved in the water.

At the same time, the different harvesting protocols also had no effect on the nitrite concentration in the water, i.e., there was no significant difference between the treatments.

3.2.3. Effect of the Factors Examined on the Nitrate Concentration in Water

Figure 4 shows the changes in nitrate concentration as a function of each factor. Time influenced the change in the concentration of nitrate dissolved in water. After the first week of the experiment, the concentration began to decrease, and then began to increase in the fourth week. The value in the fourth week was already higher than that in the first week, and the value in the fifth week was even higher. There was no change in the sixth and seventh weeks. The values in the eighth and ninth weeks were the same as those in the first week.

Of the sampling locations, the nitrate content of the samples taken after the physical filter was lower than that of the samples taken after the fish and plant cultivation units. This can be attributed to the effect of the bacterial film and algae that formed on the clay balls [48,49]. These biofilms in aquaponics settings are capable of the accumulation, and removal of nutrients through interactions between bacteria and algae [50].

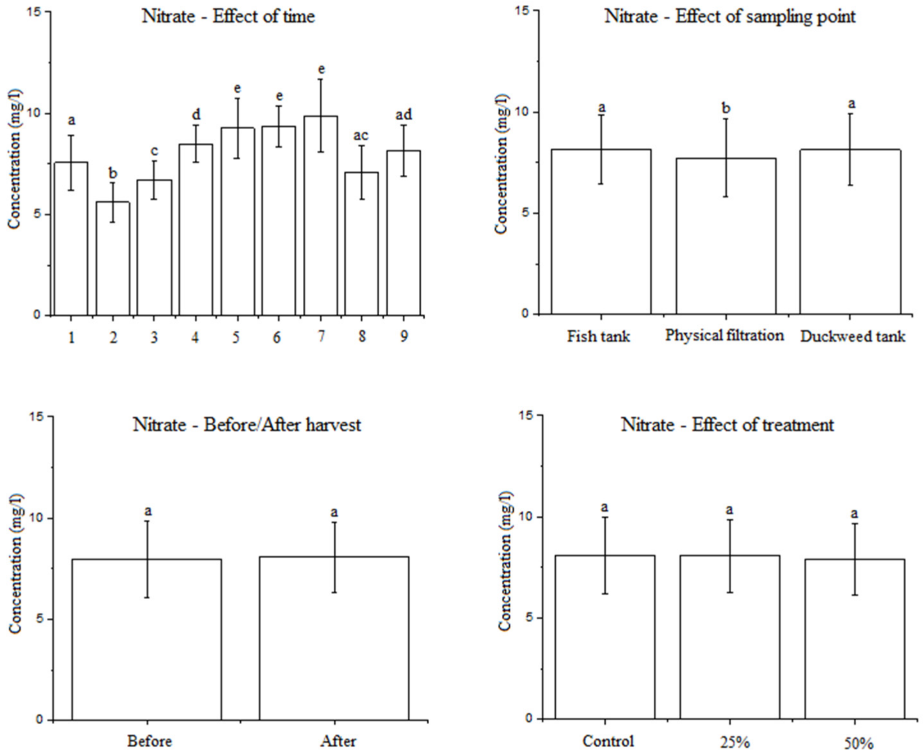

Figure 4. Effect of the examined factors on the change in nitrate concentration. The results are averages and the standard deviation for a given average. The values indicate concentration. The different letters indicate significant differences ($p < 0.05$).

Harvesting had no effect on concentration. Thus, the value can be considered constant before and after harvesting.

The different harvesting protocols also had no effect on the concentration of nitrate dissolved in the water.

3.2.4. Effect of the Factors Studied on the Orthophosphate Concentration in Water

Figure 5 shows the change in orthophosphate concentration over time during the study period, considering various factors (sampling location, time, treatments, and harvest). Based on the results, the orthophosphate concentration fluctuated throughout the study period. The lowest values were observed in the first, fifth, seventh, and eighth weeks, while higher concentrations were measured in the other weeks. This suggests that the amount of orthophosphate changed periodically, but did not show a clear, continuously increasing or decreasing trend.

A comparison of the different sampling locations revealed that significantly higher orthophosphate concentrations were measured in the effluent from the physical filter than in the fish-rearing or plant-growing units. However, there was no difference between the fish and plant cultivation units, with lower values typical in both units compared to in the physical filter. This difference may indicate that orthophosphate released from the filter or less bound there increases the measured concentration, while lower values are likely to be measured in the fish and plant units due to increased utilization by microorganisms and plants.

The studies also showed that neither harvesting events nor the treatments applied had a measurable effect on changes in orthophosphate concentration. This suggests that fluctuations in concentration are primarily influenced by internal processes within the system (microbial activity, filter media characteristics, possible accumulations and mobilizations) and not directly by the treatments applied in the experiment.

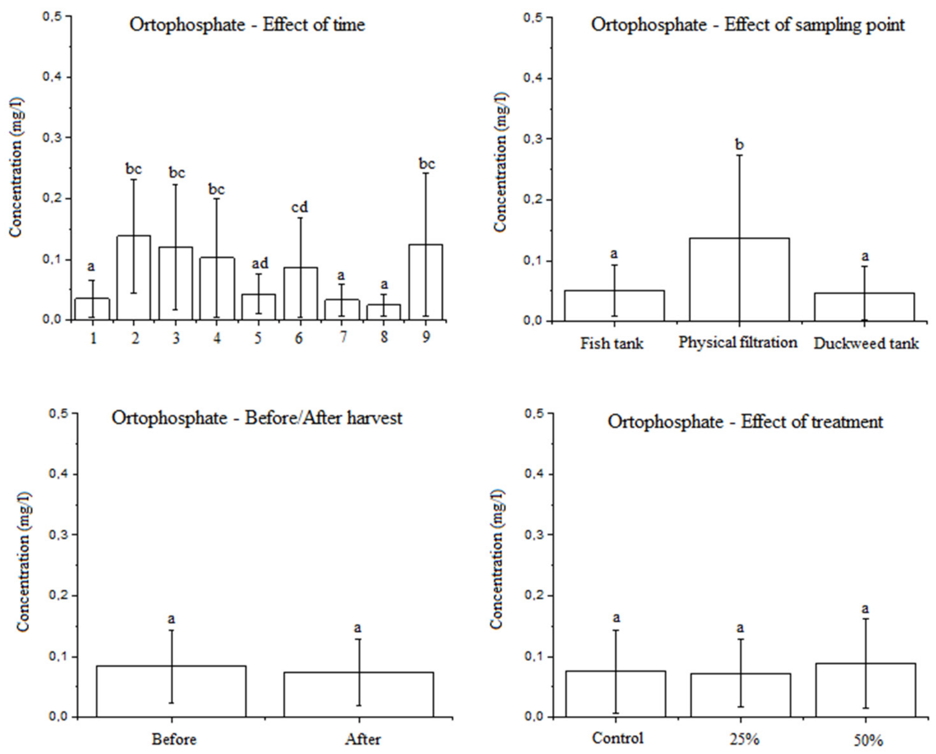

Figure 5. Effect of the factors studied on changes in orthophosphate concentration. The results are averages and standard deviations for a given average. The values indicate concentration. The different letters indicate significant differences ($p < 0.05$).

3.3. Growth of Duckweed Biomass

Figure 6 shows the daily growth rate. The control group (138%/day) achieved lower values than the groups where part of the duckweed biomass was harvested regularly (25% group—159%/day, 50% group—161%/day). Since these groups achieved greater growth and thus greater biomass during the experimental period, it is self-evident that the growth rate was also higher in these groups.

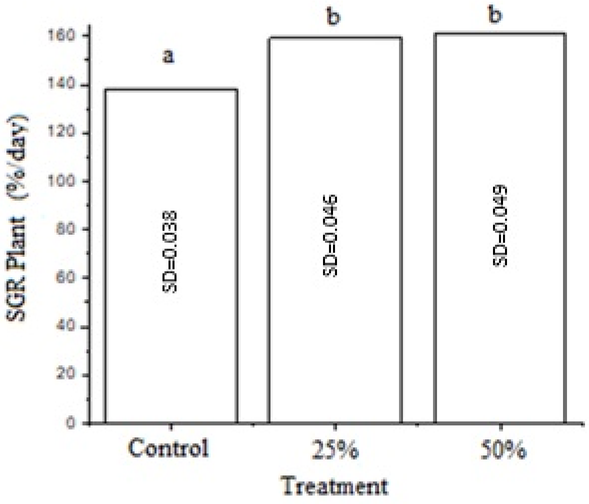

Figure 6. Effect of treatment on the daily growth rate of duckweed. The results show averages and standard deviations for each average. The different letters indicate significant difference ($p < 0.05$). SD = Standard Deviation.

In the groups where we harvested regularly, we achieved greater biomass by the end of the experimental period, but there was no difference between the 25% and 50%

groups (Figure 7). Based on the results, it can be stated that regular harvesting of duckweed had a positive effect on plant biomass development. According to [51], an increase in the concentration of ammonium ions (NH_4^+) and ammonia (NH_3^-) can have a negative effect on duckweed growth. In the control group, the ammonia concentration was higher (Figure 7), which, without regular harvesting, may explain the lower biomass growth.

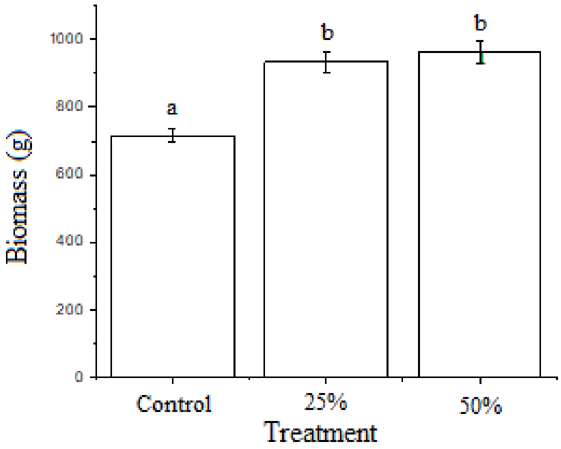

Figure 7. Effect of treatments on harvested duckweed biomass. The results show averages and standard deviations for each average. The different letters indicate significant difference ($p < 0.05$).

4. Discussion

4.1. Fish Growth and Production Parameters

Data from the literature prove that duckweed (*Lemna minor*) can be integrated into aquaculture production technology as a biological filter [52]. Based on the results of our study, it can be concluded that the use of different duckweed harvesting protocols did not have a significant effect on fish production parameters. Survival, specific growth rate (SGR), feed conversion ratio (FCR), biomass growth, and fish stock homogeneity (CV%) showed similar values for all treatments. In the experiment by [45], which examined the effect of plant biomass harvesting rate under aquaponic conditions, there was also no difference in production parameters between carp stocks of the same age and nearly identical initial weight at the end of the experiment. This suggests that the integration of duckweed into aquaculture systems is a safe and viable addition that does not compromise the efficiency and profitability of fish farming. In practice, this means that duckweed can be incorporated in a sustainable and environmentally friendly way without negatively affecting production results. However, it should be noted that according to a study by [53], depending on water temperature and feed intensity, the daily growth rate of c. carp fry in laboratory conditions can vary between 0.71 and 3.32%/day, while according to [54], the same value ranges between 0.77 and 2.35%/day of juvenile carp (5–10 g) reared individually in laboratory cement pit system. While in the experiment of [55] values around 0.5–0.6 were achieved with juvenile common carp, in controlled closed-system conditions, depending on the temperature. In the present experiment, this was 0.92%/day, which falls into the interval mentioned in other studies.

4.2. The Effect of the Factors Studied on the Ammonia Concentration in Water

During the experiment, we observed that the ammonia concentration was influenced by time, sampling location, and duckweed harvesting treatments. As time progressed, with continuous feeding and no water exchange during the experimental period, the ammonia level in the water gradually increased, especially in the third, fourth, and seventh weeks. Among the sampling locations, we measured lower ammonia levels at the physical filter compared to the duckweed and fish farming units, suggesting that the filter medium and the microbial community that developed there may have contributed in part to the reduction in ammonia. In addition, regular harvesting of duckweed also resulted in lower ammonia levels, as the free water surface available to the plants after harvesting allowed for more intensive growth, enabling them to absorb more organic matter and ammonia. Our results therefore support the observations of [47,56] that duckweed prefers ammonia among nitrogen forms and that harvesting can be an effective method for controlling ammonia levels in water. In contrast, in the experiment by [52], the ammonia concentration in the water fluctuated during the study period.

4.3. The Effect of the Factors Studied on the Nitrite Concentration in Water

During the experiment, only time had a significant effect on nitrite concentration. Nitrite levels were highest in the first week, then decreased in the second week and reached their lowest value in the third week, followed by fluctuations until the end of the experiment. In contrast, the sampling location, duckweed harvesting, and different treatment protocols did not affect nitrite levels, and there were no significant differences between treatments based on the measurements. These results show that the dynamics of nitrite concentration are primarily determined by the internal biochemical processes of the system and temporal development, while treatments such as duckweed harvesting have no direct effect. According to several studies [47,57,58], ammonium is the preferred form of nitrogen for plants. Thus, it can be said that duckweed does not prefer nitrite among the forms of nitrogen.

4.4. The Effect of the Factors Studied on the Nitrate Concentration in Water

Based on the results of the experiment, time had the greatest effect on the nitrate concentration in the water. After the first week of the experiment, the nitrate level began to decrease, then increased from the fourth week, reaching its highest value in the fifth week. It remained unchanged in the sixth and seventh weeks, while the values in the eighth and ninth weeks returned to the level of the first week. The sampling location also influenced the amount of nitrate: samples taken after the physical filter showed lower concentrations than those taken from the fish and plant cultivation units, which can be explained by the nitrate-absorbing capacity of the bacterial film and algae formed on the clay balls. In contrast, duckweed harvesting and different treatment protocols did not affect nitrate concentration. Several studies have shown that the preferred form of nitrogen for plants is ammonia [57,58]. Furthermore, in some studies nitrate dissolved in water only began to decrease after ammonium depletion [47].

4.5. The Effect of the Factors Studied on the Orthophosphate Concentration in Water

The orthophosphate concentration fluctuated during the study period, as in the study by [52], but in contrast to the study by [59], a continuous increase was observed. In the present study the lowest values were measured in the first, fifth, seventh, and eighth weeks. The sampling location influenced the amount of orthophosphate: significantly higher concentrations were measured in the effluent of the physical filter, while lower but similar values were measured in the fish and plant cultivation units. This suggests that orthophosphate released from the filter or less bound there increases the measured concentration, while in the fish and plant cultivation units, the use of orthophosphate by microorganisms and plants results in lower levels. Another explanation may be that duckweed can accumulate large amounts of phosphorus in its body [60], thus absorbing it from the water, which may cause the reduced orthophosphate content measured in the two units. In contrast, the treatments applied did not affect orthophosphate concentrations, suggesting that internal processes within the system, such as microbial activity and filter media characteristics [61], are primarily responsible for the fluctuations.

4.6. Growth of Duckweed Biomass

The results of the experiment showed that regular duckweed harvesting had a positive effect on biomass production and daily growth rate, which is consistent with the literature data [62–65].

By the end of the experimental period, the harvested groups had accumulated significantly more biomass than the control group, but there was no significant difference between the 25% and 50% harvest rates. The lower biomass and growth rate observed in the control group can be explained by the higher ammonia concentrations measured there, which, according to the literature [51], can inhibit duckweed growth. In our experiment, the control group achieved a yield of 17.9 t/ha/year, while the regularly harvested treatments achieved yields of 23.4–24 t/ha/year. These values are well in line with the wide range of data reported in the literature (10–106 t/ha/year; [66–68]). The daily growth rate also confirmed that regular harvesting promotes more dynamic duckweed growth and thus increases biomass production. Based on our results, the regular harvesting of duckweed is a simple and effective method of increasing biomass yield without significantly increasing production costs. In the present experiment, we achieved higher yields in the groups where harvesting took place, thus removing more nitrogen from the water, and thereby improving the water quality of the aquaculture system, as has been shown in other studies on IMTA systems [52].

The biomass of duckweed may be suitable for human consumption [69] and can also be used as feed [70,71], even for fish and other aquatic organisms [72,73], thus enabling circular economic solutions in closed systems. In some cases, it can also have a beneficial effect on the composition and fatty acid profile of animal products [74]. The integration of this plant into aquaculture systems therefore not only has environmental benefits but can also make farms more sustainable from an economic point of view, as it provides a more economical alternative for wastewater treatment [75]. Duckweed (*Lemna* spp.) is a fast-growing, high-protein aquatic plant that can absorb significant amounts of nitrogen and phosphorus from the water. It can therefore contribute to improving water quality, reducing nutrient accumulation, and preventing eutrophication. Its use may be particularly relevant in integrated multitrophic aquaculture (IMTA) systems, where organisms at different trophic levels utilize each other's by-products, promoting material- and energy-efficient production [76].

Based on a practical wastewater treatment context, the nutrient concentrations in the water directly reflect the uptake and removal efficiency of duckweed. Although the mineral composition of the biomass was not analyzed in present study, the decrease in dissolved nutrient concentrations provides a reliable indirect measurement of nutrient assimilation and removal performance. This approach allowed for an assessment of the influence of harvesting frequency on nutrient dynamics without requiring plant analysis. In the future, direct nutrient analysis of plant biomass will further strengthen these claims.

Author Contributions: Conceptualization, P.I.M., M.F. and P.B.; methodology, P.I.M., M.F. and P.B.; validation, P.I.M., M.F., J.P.; formal analysis, P.I.M., M.F. and J.P.; investigation, P.I.M. and B.C.B.; resources, P.B. and M.F.; data curation, P.I.M., M.F. and J.P. writing—original draft preparation, P.I.M., M.F. and P.B.; writing—review and editing, P.I.M., M.F. and P.B.; visualization, P.I.M. and M.F.; supervision, M.F. and P.B.; project administration, P.I.M. and M.F.; funding acquisition, P.B. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by University of Debrecen Program for Scientific Publication.

Institutional Review Board Statement: The ethical review committee which approved the use of animals in this study is the University of Debrecen Workplace Animal Welfare Committee. The Aquaculture Laboratory of the Faculty of Agricultural and Food Sciences and Environ-mental Management at the University of Debrecen's ethical license number is the following: 7/2025/DEMÁB (approval date: 17 September 2025).

Data Availability Statement: The raw data supporting the conclusions of this article will be made available by the authors on request.

Conflicts of Interest: The authors declare no conflicts of interest.

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