Acta Nat. Sci. (2023) 4(2): 114-125
DOI: 10.29329/actanatsci.2023.354.2
e-ISSN: 2718-0638

Original Research Paper
info@actanatsci.com
www.actanatsci.com

ACTA NATURA ET SCIENTIA PRENSİP

Impact of Different Nitrogen Sources and Concentrations on the Growth and Biochemical Structure of *Lemna minor*

Hatice Tekoğul[1]✉ • Hülya Eminçe Saygı[1] • Aysun Kop[1] • Yaşar Durmaz[1] • Ayberk Bayrak[1]

[1] Ege University, Faculty of Fisheries, Department of Aquaculture, İzmir, Türkiye, hatice.tekogul@ege.edu.tr, hulya.saygi@ege.edu.tr, aysun.kop.firat@ege.edu.tr, yasar.durmaz@ege.edu.tr, ayberkbayrak@outlook.com

✉ Corresponding Author: hatice.tekogul@ege.edu.tr

Please cite this paper as follows:
Tekoğul, H., Sayğı, H. E., Kop, A., Durmaz, Y., & Bayrak, A. (2023). Impact of Different Nitrogen Sources and Concentrations on the Growth and Biochemical Structure of *Lemna minor*. *Acta Natura et Scientia*, *4*(2), 114-125. https://doi.org/10.29329/actanatsci.2023.354.2

ARTICLE INFO

Article History
Received: 02.05.2023
Revised: 10.06.2023
Accepted: 20.06.2023
Available online: 14.08.2023

Keywords:
Lemna minor
Nitrogen sources
Concentration
Growth
Protein
Chlorophyll-*a*
Carotene

ABSTRACT

This study aimed to examine the impact of various nitrogen sources and concentrations on the growth and biochemical composition of *Lemna minor*. Specifically, three nitrogen sources, namely ammonium, nitrate, and urea, were utilized. These nitrogen sources were incorporated into the Hoagland nutrient medium at two different concentrations: 2500 µM L^{-1} and 5000 µM L^{-1}. The impact of various nitrogen concentrations on the biochemistry of *L. minor*, including the number of individuals, chlorophyll-*a* levels, carotene content, dry matter, and protein content was examined. The experimental results revealed that the 7th, 5th, and 6th groups exhibited the highest relative frond number, while no significant statistical difference ($p>0.05$) was observed between the 5000 µM L^{-1} and 2500 µM L^{-1} groups among all experimental groups. The 2nd, 7th, and 5th groups displayed the highest relative growth rate. The 4th group using NH_4-N as the source exhibited the highest total carotene and chlorophyll-*a* content. Although there were no significant differences in the dry matter and protein values of *L. minor*, the protein ratio was higher in the 3rd and 4th groups with NH_4-N as the source compared to the other groups. The results indicate that NO_3 nitrogen is the most suitable nitrogen source for promoting the growth and biochemical composition of *L. minor*, as evidenced by an increase in relative frond number and relative growth. On the other hand, NH_4 nitrogen showed favorable effects on protein, carotene, and chlorophyll-*a* content. Additionally, the experimental groups with a nitrogen concentration of 2500 µM L^{-1} yielded better overall results. Interestingly, in terms of protein efficiency, it was observed that nitrogen concentrations played a more significant role than nitrogen sources, and groups with lower dilution rates exhibited superior outcomes.

INTRODUCTION

Three quarters of the earth surface is covered by water and aquatic plants photosynthesize much more than terrestrial plants using the carbon dioxide in the air. Considering that two thirds of the photosynthetic carbon in the world is produced by algae, they are very useful organisms for the ecosystem (Carpenter & Lodge, 1986; Wersal & Madsen, 2012; Chapman, 2013; Beer et al., 2014; Madsen, 2023). At the same time, aquatic plants are the primary producers in wetlands (Yılmaz, 2004; Foundation for Water Research (FWR), 2015; Bütünoğlu, 2018). As aquatic plants enrich their production areas, they also enrich their own bodies and transform dissolved substances in water into high quality products (Madsen et al., 2001; Bütünoğlu, 2018).

Lemna species which are floating aquatic plants, are seen in many regions around the world. They are found in lakes, canals, ponds and many aquatic environments (Chaturvedi et al., 2003). *Lemna minor* is a species rich in nutrients, vitamins-minerals and pigments (Rataj & Horeman 1977; Leng et al., 1995; Madsen, 2009; Rooijakkers, 2016; Appenroth et al, 2017; Sońta et al., 2019). This plant, also found in wetlands in Türkiye, is quite prevalent, thriving in fresh waters all year round. There are 2 genera and 5 species belonging to this subfamily (Leblebici, 2010; Coşkun et al., 2018). *L. minor*, which has a very high reproductive rate, grows and reproduces asexually via the photosynthesizing and budding of young plants formed in a meristematic region at the base of the leaves, and forms a new leaf (individual). Each leaflet can produce a large number of female buds (Saygıdeğer, 1996). *Lemna* which is very tolerant to environmental conditions, can be easily cultivated at 20-30°C at a pH range of 4.5-8.5 (Topal et al., 2011). *L. minor*, whose buds develop under water in winter, are cold-resistant and start reproducing at favorable temperatures, such as in spring when normal conditions are restored (Saygıdeğer, 1996, 1997; Körner et al., 1998; Saygıdeğer et al., 2013). This species, whose growth and development are rapid in stagnant waters, is a dominant plant in the region (Akel, 2006).

Researchers have stated that nitrogen sources are one of the most important factors affecting the growth and biochemical composition of aquatic plants (Gökyay & Balcıgil, 2017, Bütünoğlu, 2018). Nitrogen is the main growth-limiting element after carbon. (Skillicorn et al., 1993; Wett & Rauch, 2003). Nitrogen, which is found in plants at a rate of 2-4%, is included in the structure of amino acids, proteins and nucleic acids. Both NO_3 and NH_4, the most important limiting nutrients for aquatic plant growth, are taken up and metabolized by the plant. The general condition and biochemical composition of the plant depends on nitrogen uptake among other factors (Wanapat, 1994; Cedergreen & Madsen, 2002).

The most important nitrogen sources that can be used by plants in production are KNO_3 (Potassium Nitrogen), NO_3-N (Nitrate Nitrogen), NH4+-N (Ammonium Nitrogen) and $(NH_2)_2CO$-N (Urea Nitrogen) (Karaşahin, 1998; Kara, 2006; Brentrup & Palliere, 2010; Bütünoğlu, 2018). *L. minor* has the capacity to take up significant amounts of inorganic N through both roots and leaves (Cedergreen & Madsen, 2002). However, the only nitrogen source is nitrate and different studies show preferential uptake of ammonium over nitrate (Caicedo et al., 2000; Cedergreen & Madsen, 2002; Fang et al., 2007; Wang et al., 2014). However, Petersen et al. (2021) reported that little was known about the effect of different nitrate-ammonium ratios on the growth rate and nutrient composition of duckweed.

Cultivation of highly nutritious aquatic plants such as *L. minor* means that relative growth can be easily increased, resulting in higher yields over a shorter period of time and thus cost reduction. This study investigated the effects of different nitrogen sources at different concentrations on plant growth and biochemical values.

MATERIAL AND METHODS

The species considered in this study is *Lemna minor* (Linneaus 1753) from the family Lemnaceae of the order Arales. *L. minor* is a free-swimming aquatic plant with a small leaf-shaped leaf and a root below the leaf. It is colored in different shades of green, 1.5-5.0 mm in size and elliptical oval shape (Figure 1).

Figure 1. *Lemna minor* used in the experiment (Original)

The *L. minor* used in the experiments was obtained from the Aquatic Plant Cultivation Laboratory of Ege University Fisheries Faculty, Urla Research Unit Laboratories.

Culture Medium and Experimental Design

The Hoagland nutrient medium, one of the culture media of *L. minor*, which is widely used in aquatic plant studies, contains 5000 µmol L^{-1} of nitrogen in 0.202 g L^{-1} (×2.5 mL) of 2M KNO_3 compound (Table 1). In this nutrient medium, KNO_3 was replaced by four different nitrogen sources with the same molar weight (Sodium nitrate, Ammonium chloride, Potassium nitrate and Urea). Two different concentrations of these nitrogen sources (2500 µM L^{-1} and 5000 µM L^{-1}) were prepared and a total of eight experimental groups were studied. (Table 2).

Table 1. Hoagland nutrient medium

Component	Stock Solution	mL Stock Solution 1L^{-1}
Macronutrients		
2M $MgSO_4 \bullet 7H_2O$	493 g L^{-1}	1
2M $Ca(NO_3)_2 \bullet 4H_2O$	236 g 0.5L^{-1}	2.5
1M KH_2PO_4 (pH to 6.0)	136 g L^{-1}	0.5
2M KNO_3	202 g L^{-1}	2.5
1M NH_4NO_3	80 g L^{-1}	1
Iron (Sprint 138 Iron Chelate)	15 g L^{-1}	1.5
Micronutrients		
H_3BO_3	2.86 g L^{-1}	1
$MnCl_2 \bullet 4H_2O$	1.81 g L^{-1}	1
$ZnSO_4 \bullet 7H_2O$	0.22 g L^{-1}	1
$CuSO_4 \bullet 5H_2O$	0.051 g L^{-1}	1
$H_3MoO_4 \bullet H_2O$ or	0.09 g L^{-1}	1
$Na_2MoO_4 \bullet {}_2O$	0.12 g L^{-1}	1

1-liter containers were used in the experiments. The water volume was kept at 250 mL and 5 cm in height and at the end of the study it was 4.5 cm due to evaporation. The water used in the research was passed through 1-10 µm, activated carbon, UV and softening filter and pH was adjusted to 6.5-7. The cultured samples were kept constant at 25±1°C with central heating. The temperature was measured with the help of a thermometer with ±0.1°C accuracy. The prepared stock solutions and the culture media prepared from them were sterilized in an autoclave at 121°C for 20 minutes. Ph values were measured using Orion branded pH meter. Oxygen was measured with a WTW Wissenschaftlich Oxi 315i/SET oxygen meter.

Table 2. Nitrogen sources and concentrations of the experimental groups

Experimental Groups	Nitrogen Sources	Concentration µM L^{-1}
1	$NaNO_3$	5000
2	(Na Nitrate-N)	2500
3	NH_4Cl	5000
4	(Ammonium-N)	2500
5	KNO_3	5000
6	(K Nitrate-N)	2500
7	$(NH_2)_2CO$	5000
8	(Urea-N)	2500

Day-night (16 hours light and 8 hours dark photo period) period was used in the experiments. Daylight led lamps were used to illuminate the system and the light intensity was measured as 216 µmol m^{-2} s^{-1} with a light meter.

Enrichment medium was provided at the beginning of the experiments. A new medium was added once a week and the experiments continued for 15 days with three repetitions.

Determination of Relative Frond Number and Relative Growth

As far as the relative number of fronds is concerned, the number of fronds in each experimental group was counted every day. At the end of the study, 250 mL volume samples in each experimental group

were harvested at the end of the study to determine the relative growth obtained. The weights of the *Lemnas*, whose weights were measured at the beginning, were measured at the end of production to determine the relative growth. After the leaves were removed from the water with the help of paper towels, their wet weights were measured and recorded on an electronic precision balance. Relative frond number and relative growth rate were calculated as given in Table 3 (Wang et al., 2014).

Table 3. Definitions, formulas and units of relative growth rate by weight and frond number

Definition	Formula	Volume
Relative Frond Number Rate	$\frac{ln(N_1) - \ln(N_2)}{t_2 - t_1}$	day^{-1}
Relative Growth Rate	$\frac{ln(W_1) - \ln(W_2)}{t_2 - t_1}$	g.g^{-1}day^{-1}

Note: N: Frond Number; W: Weight; N_1: Initial Frond Number; N_2: Last Frond Number; W_1: Initial Weight; W_2: Final Weight; t: Time

Chemical Analyses

Total Carotene and Chlorophyll-a Analysis

The wet mass obtained was kept in a deep freezer at -25°C for 24 hours and frozen and then dried in an oven at 30°C for 48 hours.

Total carotene and chlorophyll-*a* amounts were measured using the spectrophotometric method. For this purpose, 5 mg of dried sample was taken and treated with 5 mL of methanol (Merck 100%, Germany) and homogenized with a Hettich homogenizer for 5 minutes. It was then subjected to ultrasound (BandelinSonorex Super RK102H) bath at 70°C for 10 minutes. After the extract was separated by centrifuge at 3500 rpm, the samples were read at wavelengths of 475 nm for total carotene and 666 nm for chlorophyll-*a* on an Optima SP3000 Nano uv-vis spectrophotometer. Total carotene and chlorophyll-*a* amounts were determined by the formulas given below in Equation 1 (Zou & Richmond, 2000) and Equation 2 (Sanchez et al., 2005).

$$C_{Carotene}\ (mg\ g^{-1}) = 4.5.\ A_{475} \quad (1)$$

where A_{475} is absorbance value read at 475 nm.

$$C_{Chlorophyll\text{-}a}\ (mg\ g^{-1}) = 13.9.\ A_{666} \quad (2)$$

where A_{666} is absorbance value read at 666 nm.

Dry Matter

Dry matter analysis was performed according to AOAC (1990) (934.01). The results were calculated using the following formula.

$$DM\% = \frac{\text{Dried sample weight (g)}}{\text{Sample weight included in the analysis(g)}} \times 100 \quad (3)$$

Crude Protein

Crude protein analysis (AOAC-976.05) was performed according to the method (AOAC, 1990) and calculated according to the formula below.

$$\text{Crude Protein} = \frac{(V_0 - V_1)xcx.0.014x6.25}{m} \quad (4)$$

where V_0 = HCl volume used in the blind test (ml); V_1 = volume of HCl used in sample titration (ml); c = HCl concentration (mol/l); m = Weight of the sample (g).

Statistical Analysis

The experiments were carried out with three replications. Mean and standard deviation were calculated for initial and final weights, relative frond number, relative growth, carotene, chlorophyl-a, dm and protein at different nitrogen sources (Mean±SD) and differences between different nitrogen sources were tested for one-way analysis of variance at 0.05 level of significance. In order to fulfill the assumptions of the analysis of variance. Levene's test was used to test the homogeneity of variances and the Kolmogorov-Smirnov test was used to test the normality assumption. Since the assumptions were fulfilled, one-way analysis of variance (ANOVA) followed by Duncan test was used to reveal the difference in means (Sokal & Rohlf, 1995). Furthermore, the differences for different molarities (2500 μM L^{-1} and 5000 μM L^{-1}) in different nitrogen groups were analyzed by t-test for the significance of the difference between two means. A significance level of 0.05 was taken into account in the statistical

evaluation of all these data and IBM SPSS 25.0 and Microsoft Excel 2016 software were used.

RESULTS

As seen in Table 4, the relative frond number increase of *L. minors* grown under different nitrogen sources was the highest in the 7th experimental group i.e., 0.079±0.007% leaves day-^{1}, while the lowest was 0.068±0.011% leaves $^{-1}$ in the 2nd experimental group.

The increase in the number of fronds across all experimental groups was in direct proportion the concentrations. However, there was no statistical difference between the changes in the number of individuals in all experimental groups ($p>0.05$).

There was no statistical difference in the initial weight ($p>0.05$) and final weight ($p>0.05$) of *L. minor* with different nitrogen sources (Table 4).

The highest relative frond number was determined in the 7th, 5th and 6th experimental groups (Figure 2). When evaluated in terms of relative frond number ratio, there was no statistical difference in 5000 µM L^{-1} and 2500 µM L^{-1} groups of all experimental groups ($p>0.05$).

Table 4. Biometric, nutrient and pigment content in the experimental groups (DM: Dry matter, $\bar{X}$±SD: Mean±Standard deviation)

Nitrogen Sources	Concentration (µM L^{-1})	Initial Weight ($\bar{X}$±SD)	Final Weight ($\bar{X}$±SD)	Relative Frond Number (% individual. day^{-1}) ($\bar{X}$±SD)	Relative Growth Weight % ($\bar{X}$±SD)	Carotene mg 100 mL^{-1} ($\bar{X}$±SD)	Chlorophyll-*a* mg 100 mL^{-1} ($\bar{X}$±SD)	KM% ($\bar{X}$±SD)	Protein Values (%)
$NaNO_3$ (Na Nitrate-N)	5000	2.25±0.01	3.53±0.49	0.070±0.012	1.19±0.21	133.33±0.67^{d}	532.67±1.20^{d}	9.4±1.82	18.45
$NaNO_3$ (Na Nitrate-N)	2500	2.23±0.02	4.83±0.14	0.068±0.011	1.520.04	110.00±0.57^{c}	442.67±0.33^{c}	11.9±4.45	16.84
NH_4Cl (Ammonium-N)	5000	2.34±0.11	3.37±0.29	0.070±0.011	1.150.11	139.67±0.33^{e}	552.67±1.45^{e}	11.6±3.23	21.32
NH_4Cl (Ammonium-N)	2500	2.27±0.04	3.57±0.09	0.069±0.013	1.22±0.04	144.67±0.67^{f}	578.67±0.67^{f}	12.1±1.29	18.10
KNO_3 (K Nitrate-N)	5000	2.30±0.04	4.12±0.55	0.074±0.012	1.35±0.18	94.67±0.33^{a}	376.67±0.88^{a}	9.8±1.94	18.04
KNO_3 (K Nitrate-N)	2500	2.33±0.09	3.89±0.77	0.071±0.011	1.26±0.31	99.33±0.33^{b}	397.00±1.00^{b}	11.6±2.44	17.67
$(NH_2)_2CO$ (Urea-N)	5000	2.25±0.02	4.14±0.37	0.079±0.007	1.35±0.13	111.00±0.00^{c}	436.33±1.33^{c}	11.0±2.00	17.52
$(NH_2)_2CO$ (Urea-N)	2500	2.22±0.01	3.19±0.05	0.069±0.006	1.11±0.02	140.00±2.08^{e}	530.33±7.84^{d}	11.4±3.89	18.19

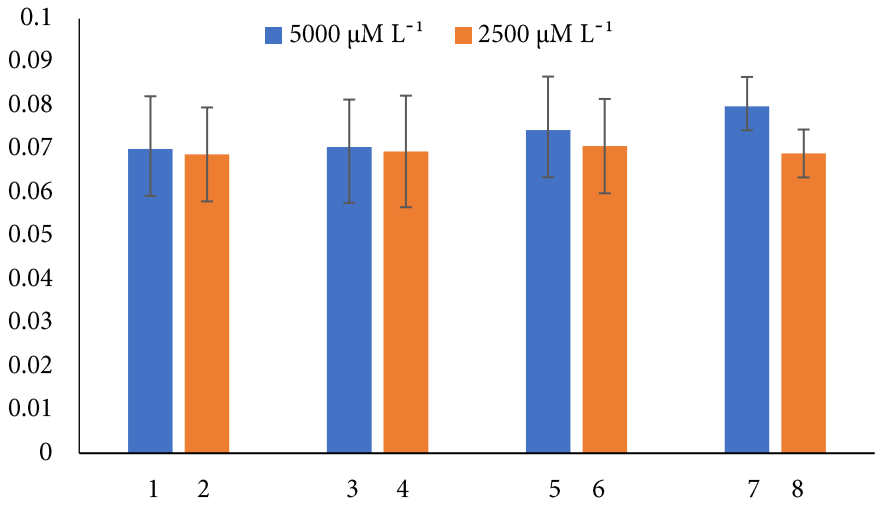

Figure 2. Relative frond number rate of the experimental groups

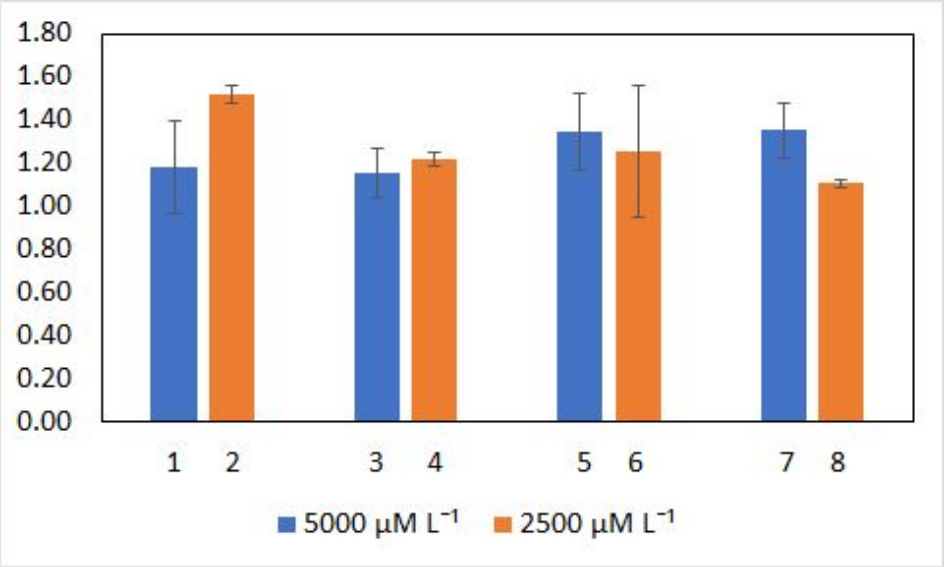

Figure 3. Relative growth rate of the experimental groups

When the experimental groups were analyzed in terms of relative growth rate, no statistical difference was found between the 3rd-4th and 5th-6th experimental groups ($p>0.05$). In addition, there was a statistical difference ($p\leq0.05$) in trial groups 1-2 and 7-8. The highest relative growth rate was observed in the group with 2500 µM L^{-1} in groups 1 and 2, while there was a statistical difference ($p\leq0.05$) in trial groups 7 and 8 with 5000 µM L^{-1}. The highest relative growth rate was observed in 2nd, 7th and 5th experimental groups (Table 4, Figure 3).

The sample obtained from the NH_4-N source in group 4 exhibited the highest total carotene content (144.667±0.667 mg 100 mL^{-1}). Additionally, within the same group, the highest chlorophyll-*a* content was measured, amounting to 578.667±0.667 mg 100 mL^{-1}. The carotene and chlorophyll-*a* values of *L. minor* showed significant differences across different nitrogen sources and concentrations ($p\leq0.05$). However, there were no statistically significant differences observed between groups 2 and 7 in terms of carotene and chlorophyll-*a* content ($p>0.05$). Similarly, the amount of chlorophyll-*a* did not differ significantly between groups 1 and 8 ($p>0.05$) (Table 4). When the dry matter and protein values of *L. minor* grown with different nitrogen sources and concentrations were analyzed, no significant difference was observed, but the protein ratio was higher in the 3rd and 4th group trials with NH_4-N as the source compared to the other groups. The 3rd group had the highest protein rate with 21.32%, followed by the 1st group with 18.45% and then the 8th group with 18.19%. The lowest protein rate was 16.84% in group 2 (Table 4).

According to the experimental groups, the highest wet weight of the leaves was observed in groups 2, 7 and 5, while the highest dry weight was observed in groups 2, 5 and 6, respectively. In addition, the Dry Weight/Wet Weight ratio was found in the 6th, 3rd and 5th groups, respectively (Table 5).

DISCUSSION

This study aimed to investigate the impact of various culture conditions, enriched with different nitrogen sources and their respective concentrations, on the relative growth rates, relative frond number increase, carotene, chlorophyll-*a*, and protein content of *L. minor*. *L. minor* is an aquatic plant of significant interest to various industries including food, cosmetics, pharmaceuticals, among others.

The family Lemnaceae possesses the ability to assimilate nitrogen from multiple sources, including ammonium, nitrate, nitrite, urea, and certain amino acids. However, ammonium and nitrate are generally recognized as the primary nitrogen sources for most species within this family. In a previous study by Ericsson et al. (1982) investigating growth under different nitrogen concentrations, they found that the growth of Lemnaceae species was primarily driven by nitrogen demand rather than concentration ratios.

Table 5. Mean wet weight (FW) and dry weight (DW) amounts and Wet Weight/Dry Weight rate of fronds according to the experimental groups ($\bar{X}$±D: Mean ± Deviation)

Nitrogen Sources	Concentration (μM L^{-1})	Groups	Wet Weight (g) ($\bar{X}$±D)	Dry Weight (g) ($\bar{X}$±D)	Dry Weight/Wet Weight ($\bar{X}$±D)
$NaNO_3$ (Na Nitrate-N)	5000	1	3.5300±0.6975	0.1567±0.0287	0.0448±0.0047
$NaNO_3$ (Na Nitrate-N)	2500	2	4.8267±0.1960	0.2000±0.0082	0.0414±0.0000
NH_4Cl (Ammonium-N)	5000	3	3.3700±0.4090	0.1633±0.0170	0.0497±0.0104
NH_4Cl (Ammonium-N)	2500	4	3.5700±0.1219	0.1700±0.0327	0.0476±0.0092
KNO_3 (K Nitrate-N)	5000	5	4.1233±0.7757	0.2000±0.0374	0.0486±0.0037
KNO_3 (K Nitrate-N)	2500	6	3.8867±1.0817	0.1900±0.0356	0.0506±0.0066
$(NH_2)_2CO$ (Urea-N)	5000	7	4.1433±0.5196	0.1767±0.0236	0.0442±0.0123
$(NH_2)_2CO$ (Urea-N)	2500	8	3.1867±0.0634	0.1500±0.0082	0.0471±0.0029

Additionally, the study highlighted the existence of viable strategies to achieve consistent growth rates under low optimal nitrogen nutrition. Moreover, Ericsson et al. (1982) observed that *L. minor* did not uptake nitrogen from the environment in quantities sufficient to meet its metabolic requirements. Minimum and optimal nitrogen levels are thought to vary greatly between species and geographical isolates, with increasing light intensity increasing the optimal nitrogen requirements for growth. The minimum nitrogen level (in *L. miniscula*) was determined between 0.0016 mM L^{-1} and 0.08 mM L^{-1}. The maximum tolerated nitrogen level ranged from 30 mM L^{-1} (*L. miniscula*) to 450 mM L^{-1} (*L. aequinoctialis*), while the optimal nitrogen requirement ranged from 0.01 mM L^{-1}, (*Wolffia colombia*) to 30 mM L^{-1} (*Spirodela polyrrhiza*) (Landolt & Kandeler, 1987).

When all nitrogen forms were analyzed, *L. minor* and *L. gibba* were reported to prefer using nitrate nitrogen and ammonium nitrogen for growth compared to other nitrogen forms (Wang et al., 2014; Iatrou et al., 2019). However, high concentrations of ammonium ions have also been reported to inhibit duckweed growth (Oron et al., 1984).

In our study, no mortality was observed in *L. minor* leaves throughout the experiments. In the light of the data obtained, the relative frond number increase and relative growth rate were higher in the NO_3 form of the experimental groups (2, 5 and 6). Similarly, Petersen et al. (2021) reported that *L. minor* increased the relative growth rate of nitrate (75-100 mM) rich diets the most. The reason for this was that nitrate acted as a signaling molecule that rapidly triggers gene, metabolism and growth changes (Gojon et al., 2011).

In experiments with different nitrogen sources and their two different concentrations, it was found that the highest increase in relative frond number in *L. minor* groups was 0.079±0.007% individual day^{-1} in the 7th group. This value was followed by 0.074±0.012 and 0.071±0.011% individual day^{-1} in the 5th and 6th groups, respectively. The highest relative growth rate was in the experimental group 2, 7 and 5. While the final weights of the experimental groups indicate a potential increase in the relative growth rate, caution must be exercised when extrapolating this observation to the relative fond numbers.

However, it is not possible to say the same thing for relative frond numbers. In contradistinction to this study, a study conducted by Wang et al. (2014) indicated that the 28 mg L^{-1} NH_4^+-N concentrations had maximum relative dry weight growth rate (RDWGR) and relative frond number growth rate (RFNGR) values and the RFNGR and RDWGR were significantly correlated. However, upon reviewing numerous studies on nitrogen forms, it becomes evident that *L. minor* and *L. gibba* species exhibit a preference for utilizing nitrate nitrogen and ammonium nitrogen (NH_4-N) as sources for growth, as opposed to other available nitrogen forms. (Jensen et al., 2006, Brentrup & Palliere, 2010; Wang et al., 2014; Latrou et al., 2019). This result is in agreement with our study findings.

In the study conducted NH_4-N demonstrated that the highest carotene and chlorophyll-*a* were in group 4. Similarly, the study conducted by Petersen at al. (2021) reported that the NH_4+-N concentrations significantly affected the chlorophyll-*a* and carotenoid contents and the highest Chl-*a*, Chl-*b*, Chl-*a*+*b* and Car contents were in the 84 mg L^{-1} NH_4^+-N concentrations. Also, the study stated that higher (280 and 840 mg L^{-1}) or lower (2, 7 and 28 mg L^{-1}) NH_4^+-N concentrations caused a significant decrease in the Chl-*a*, Chl-*b*, Chl-*a*+*b* and Car contents. In the present study, an inverse relationship between nitrogen concentrations and carotenoid contents was observed in the experimental groups, indicating that higher nitrogen concentrations were associated with lower levels of carotenoids. It is because although NH_4^+-N is a nitrogen source chosen by most plants (von Wirén et al., 2000), it is reported to be toxic for specific plants in high-concentration medium (Rudolph & Voigt, 1986; Britto & Kronzucker, 2002; Cao et al., 2004; Boussadia et al., 2010; Wang et al., 2011, 2014; Li et al., 2013).

In the study the mean dry matter rate in *L. minor* was 8.47% (91.53% water rate). A study conducted by Skillicorn et al. (1993) reported that 92-94% of fresh plant weight is water. A study conducted by Dayıoğlu et al. (2006) reported that the plant contains 92% water. The data in our study is in agreement with the aforementioned studies.

According to a study conducted by Leng et al. (1995) the ammonium N in water affects raw protein accumulation in the plant. Another study conducted by the researcher suggests that it is possible to acquire optimal protein content in medium where Nitrogen is 60 mg N L^{-1} or higher (Leng, 1999). Similarly, a study conducted by adding ammonium nitrogen to the medium in order to increase the biomass of *L. minor* and reproduce the plant obtained a high rate of raw protein and the protein content of *L. minor* increased from 21.9% to 39.4% (Latrou et al., 2019). Also, our study found the highest protein value in group 3 whose source was NH_4-N at the level of 21.32%.

A study conducted by Culley & Epps (1973) demonstrated that there is a strongly positive correlation between highly dissolved nutrients and plant properties, especially protein and digestibility. Also, specific researchers reported that there are positive correlations between nutrition concentrations and dry matter productivity, raw protein and phosphorus content (Whitehead et al., 1987; Alaerts et al., 1996). However, Bergman et al. (2000) found a very little difference in dry matter (DM) productivity and reported that there is no difference in the protein content of *L. gibba* which is cultivated in a variety of nutritional levels (52 to 176 mg N L^{-1}).

Although there was no statistically significant difference between the groups in the study, the highest wet weight was in group 2, 7 and 5, the highest dry weight was in group 2, 5 and 6 and the highest DW/FW rate was in group 6, 3 and 5.

The study conducted by Petersen et al. (2021) observed all biggest FW and DW in the 28 mg L^{-1} NH_4^+-N concentration and all smallest ones in the 840 mg L^{-1} concentration. However, the highest DW/FW rate was in 280 mg L^{-1} concentration.

Similarly, this study obtained higher rates in the groups with a lower concentration. The study conducted by Petersen et al. (2021) reported that *L. minor* uses both ammonium and nitrate as a nitrogen source, has developed a few NO_3 intake systems to survive in the changing medium and both its roots and fronds are able to receive nitrate and ammonium from the medium. This researcher noted that higher dilution of the nutrient medium, i.e., much lower nutrient concentrations, would in any case lead to lower protein productivity.

CONCLUSION

According to the study conducted it is possible to state that *L. minor* uses all nitrogen sources and while nitrate sources come into prominence in the weight gain, ammonium nitrogen comes into prominence in the chlorophyll-*a* and carotene amount. The results show that NO_3 nitrogen is the optimal nitrogen source for the growth, leaf number, biochemical composition and growth of *L. minor*. Although NO_3 nitrogen was effective in growth and development, NH_4 nitrogen was more effective on protein, carotene and chlorophyll-*a* content. In the protein content it is possible to state that concentrations are as crucial as nitrogen source.

It is necessary to acquire a standardized product quality to use *L. minor* in food and aquatic feed. Also, the biomass and protein amount acquired is crucial for a quality product. For that purpose, it is necessary to use a standard cropping system. In addition to abiotic factors such as light intensity, light spectrum, photoperiod, temperature, water and *L. minor* movement, it is necessary to try a variety of nutritional sources and concentrations in different volumes.

ACKNOWLEDGEMENTS

This research was supported by Ege University Scientific Research Projects (BAP Project No: 2018/SÜF/011). The authors would like to thank Ege University Scientific Research Projects Department (Izmir, Türkiye) for their financial support and Ege University Faculty of Fisheries (Izmir, Türkiye) for research infrastructure.

Compliance with Ethical Standards

Authors' Contributions

HT: Manuscript design, laboratory experiment, writing, draft checking.

HS: Statistical analyses.

AK: Draft checking, reading, and editing.

YD: Draft checking, reading, and editing.

AB: Laboratory experiment.

All authors read and approved the final manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

For this type of study, formal consent is not required.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

REFERENCES

Akel, E. (2006). *Comparative studies on feeding possibilities of duckweed (Lemna minor L.) in aquarium environment of Singaporean red-cheeked freshwater turtles* (*Pseudemys scripta elegans*). [Ph.D. Thesis. Kütahya Dumlupınar University]

Alaerts, G. J., Rahman Mahbubar, MD., & Kelderman, P. (1996). Performance analysis of a full-scale duckweed-covered sewage lagoon. *Water Research*, *30*(4), 843-852. https://doi.org/10.1016/0043-1354(95)00234-0

AOAC. (1990). Official methods of analysis (pp. 1028-1039). In Helrich, K. (Ed.), *Association of Official Analytical Chemists International. 15th ed*. Association of Official Analytical Chemists Inc.

Appenroth, K. J., Sree, K. S., Böhm, V., Hammann, S., Vetter, W., Leiterer, M., & Jahreis, G. (2017). Nutritional value of duckweeds (Lemnaceae) as human food. *Food Chemistry*, *217*, 266-273. https://doi.org/10.1016/j.foodchem.2016.08.116

Beer, S., Björk, M., & Beardall, J. (2014). *Photosynthesis in the marine environment*. Wiley-Blackwell.

Bergmann, B. A., Cheng, J., Classen, J., & Stomp, A. -M. (2000). In vitro selection of duckweed geographical isolates for potential use in swine lagoon effluent renovation. *Bioresource Technology*, *73*(1), 13-20. https://doi.org/10.1016/S0960-8524(99)00137-6

Boussadia, O., Steppe, K., Zgallai, H., El Hadj, S. B., Braham, M., Lemeur, R., & Van Labeke, M. C. (2010). Effects of nitrogen deficiency on leaf photosynthesis, carbohydrate status and biomass production in two olive cultivars 'Meski'and 'Koroneiki'. *Scientia Horticulturae*, *123*(3), 336-342. https://doi.org/10.1016/j.scienta.2009.09.023

Brentrup, F., & Pallière, C. (2010). Nitrogen use efficiency as an agro-environmental indicator. *Proceedings of the OECD Workshop on Agrienvironmental Indicators: Lessons Learned and Future Directions*, Switzerland, pp. 1-9.

Britto, D. T., & Kronzucker, H. J. (2002). NH_4^+ toxicity in higher plants: A critical review. *Journal of Plant Physiology*, *159*(6), 567-584. https://doi.org/10.1078/0176-1617-0774

Bütünoğlu, A. (2018). *Evaluation of nutrient removal by floating wetland and aquatic plants in water resources.* [MSc. Thesis. Ankara TR Ministry of Agriculture and Forestry, General Directorate of Water Management].

Caicedo, J. R., van der Steen, N. P., Arce, O., & Gijzen, H. J. (2000). Effect of total ammonia nitrogen concentration and pH on growth rates of duckweed (*Spirodela polyrrhiza*). *Water Research*, *34*(15), 3829-3835. https://doi.org/10.1016/S0043-1354(00)00128-7

Cao, X., Ma, L. Q., & Tu, C. (2004). Antioxidative responses to arsenic in the arsenic-hyperaccumulator Chinese brake fern (*Pteris vittata* L.). *Environmental Pollution*, *128*(3), 317-325. https://doi.org/10.1016/j.envpol.2003.09.018

Carpenter, S. R., & Lodge, D. M. (1986) Effects of submersed macrophytes on ecosystem processes. *Aquatic Botany*, *26*, 341-370. https://doi.org/10.1016/0304-3770(86)90031-8

Cedergreen, N., & Madsen, T. V. (2002). Nitrogen uptake by the floating macrophyte *Lemna minor*. *New Phytologist*, *155*(2), 285-292. https://doi.org/10.1046/j.1469-8137.2002.00463.x

Chapman, R. L. (2013). Algae: The world's most important "plants"—an introduction. *Mitigation and Adaptation Strategies for Global Change*, *18*(1), 5-12. https://doi.org/10.1007/s11027-010-9255-9

Chaturvedi, K. M. M., Langote, D. S., & Asolekar, R. S. (2003). *Duckweed-fed fisheries for treatment of low strength community waste water*. WWWTM Newsletter, Asian Institute of Technology, India. https://ait.ac.th/

Coşkun, Ö. F., Aydın, D., Akıska, S., Özel, H. B., & Varol, T. (2018). Determination of the duckweed species in Turkey. *Journal of Bartın Faculty of Forestry*, *20*(1), 145-151. http://doi.org/10.24011/barofd.406868

Culley Jr, D. D., & Epps, E. A. (1973). Use of duckweed for waste treatment and animal feed. *Water Pollution Control Federation*, *45*(2), 337-347.

Dayıoğlu, H., Özyurt, M. S., Aker, M. E., Çaycı, M. K., & Solak, C. N. (2006). *Pseudemys scripta elegans*'ın akvaryum ortamında *Lemna minor* L. ile besleme imkanları üzerine bir araştırma [*A study on feeding oppurtinies of Pseudemys scripta elegans fed with Lemna minor L. in aquarium*]. *Dumlupınar Üniversitesi Fen Bilimleri Dergisi*, (011), 1-10.

Ericsson, T., Larsson, C. -M., & Tillberg, E. (1982). Growth responses of *Lemna* to different levels of nitrogen limitation. *Zeitschrift für Pflanzenphysiologie*, *105*(4), 331-340. https://doi.org/10.1016/S0044-328X(82)80029-9

Fang, Y. Y., Babourina, O., Rengel, Z., Yang, X. E., & Pu, P. M. (2007). Ammonium and nitrate uptake by the floating plant *Landoltia punctata*. *Annals of Botany*, *99*(2), 365-370. https://doi.org/10.1093/aob/mcl264

Foundation for Water Research (FWR). (2015). *Toxic algal blooms in drinking water reservoirs. foundation for water research*. Retrieved on June 22, 2023, from http://www.fwr.org/drnkwatr/algaltox.htm

Gojon, A., Krouk, G., Perrine-Walker, F., & Laugier, E. (2011). Nitrate transceptor(s) in plants. *Journal of Experimental Botany*, *62*(7), 2299-2308. https://doi.org/10.1093/jxb/erq419

Gökyay, O., & Balcıgil, M. (2017). *Ham ve sentetik atıksularda su mercimeği (Lemna minor L.) kullanılarak karbon ve besi maddelerinin gideriminin incelenmesi ve karşılaştırılması* [The investigation and comparison of carbon and nutrient removal from domestic and synthetic wastewaters using duckweed (*Lemna minor* L.)]. *Marmara Fen Bilimleri Dergisi*, *29*(4), 124-130. https://doi.org/10.7240/marufbd.369743

Iatrou, E. I., Kora, E., & Stasinakis, A. S. (2019). Investigation of biomass production, crude protein and starch content in laboratory wastewater treatment systems planted with *Lemna minor* and *Lemna gibba*. *Environmental Technology*, *40*(20), 2649-2656. https://doi.org/10.1080/09593330.2018.1448002

Jensen, J., Sorokin, N., Dirven-van Breemen, E. M., Bogolte, T., Erlacher, E., Ehlers, C., Ter Laak, T., Hartnik, T., Bierkens, J., Rutgers, M., Mesman, M. (2006). A triad-based selection of tools for site-specific assessment of ecological risk (pp. 65-116). In Jensen, J., & Mesman, M. (Eds.), *Ecological risk assessment of contaminated land-Decision support for site specific investigations*. Liberation: RIVM report number 711701047. Accessed June 22, 2023, from https://www.rivm.nl/bibliotheek/rapporten/711701047.pdf

Kara, B. (2006). *Determination of nitrogen uptake and utilization efficiency of corn with different plant densities and different nitrogen doses in Çukurova conditions.* [Ph.D. Thesis. Adana Çukurova University].

Karaşahin, B. (1998). *A research on the benthic fauna of Lake Kovada and Kovada Channel*. [M.Sc. Thesis. Isparta Süleyman Demirel University]

Körner, S., Lyatuu, G. B., & Vermaat, J. E. (1998). The influence of *Lemna gibba* L. on the degradation of organic material in duckweed-covered domestic wastewater. *Water Research*, *32*(10), 3092-3098. https://doi.org/10.1016/S0043-1354(98)00054-2

Latrou, E. I., Kora, E., & Stasinakis, A. S. (2019). Investigation of biomass production, crude protein and starch content in laboratory wastewater treatment systems planted with *Lemna minor* and *Lemna gibba*. *Environmental Technology*, *40*(20), 2649-2656, https://doi.org/10.1080/09593330.2018.1448002

Leblebici, Z. (2010). *Effect of nitrate, phosphate and sulphate on accumulation of some heavy metals in members of duckweed spread in Turkey*. [Ph.D. Thesis. Kayseri Erciyes University].

Leng, R. A. (1999). *Duckweed: A tiny aquatic plant with enormous potential for agriculture and environment*. *Proceedings of the 47th International Conference on Environmental Systems*, South Carolina. pp. ICES-2017-281.

Leng, R. A., Stambolie, J. H., & Bell, R. (1995). Duckweed - a potential high-protein feed resource for domestic animals and fish. *Livestock Research for Rural Development*, *7*(1), 5.

Li, G., Li, B., Dong, G., Feng, X., Kronzucker, H. J., & Shi, W. (2013). Ammonium-induced shoot ethylene production is associated with the inhibition of lateral root formation in *Arabidopsis*. *Journal of Experimental Botany*, *64*(5), 1413-1425. https://doi.org/10.1093/jxb/ert019

Madsen, J. D. (2009). Eurasian watermilfoil. In L. A. Gettys, W. T. Haller, & M. Bellaud (Eds.), *Biology and control of aquatic plants: A best management practices handbook* (pp. 95-98). Accessed June 22, 2023, from https://plants-archive.ifas.ufl.edu/wp-content/uploads/files/mng/AERF_handbook.pdf

Madsen, J. D. (2023). Impact of invasive aquatic plants on aquatic biology. In L. A. Gettys, W. T. Haller, & D. G. Petty (Eds.), *Biology and control of aquatic plants: A Best Management Practices Handbook. 4th edition* (pp. 1-6). Aquatic Ecosystem Restoration Foundation.

Madsen, J. D., Chambers, P. A., James, W. F., Koch, E. W., & Westlake, D. F. (2001). The interaction between water movement, sediment dynamics and submersed macrophytes. *Hydrobiologia*, *444*(1), 71-84. https://doi.org/10.1023/A:1017520800568

Oron, G., Porath, D., & Jansen, H. (1987). Performance of the duckweed species *Lemna gibba* on municipal wastewater for effluent renovation and protein production. *Biotechnology and Bioengineering*, *29*(2), 258-268. https://doi.org/10.1002/bit.260290217

Petersen, F., Demann, J., Restemeyer, D., Ulbrich, A., Olfs, H. W., Westendarp, H., & Appenroth, K. J. (2021). Influence of the nitrate-N to ammonium-N ratio on relative growth rate and crude protein content in the duckweeds *Lemna minor* and *Wolffiella hyalina*. *Plants*, *10*(8), 1741. https://doi.org/10.3390/plants10081741

Rataj, K., & Horeman, T. J. (1977). *Aquarium plants-their identification, cultivation and ecology*. T.F.H. Publications, Inc.

Rooijakkers, P. (2016). *Photosynthesis model to predict duckweed growth at the Ecoferm greenhouse*. [Bachelor Thesis. Wageningen University & Research].

Rudolph, H. J., & Voigt, J. U. (1986). Effects of NH^{+}_{4}-N and NO^{+}_{3}-N on growth and metabolism of *Sphagnum magellanicum*. *Physiologia Plantarum*, *66*(2), 339-343. https://doi.org/10.1111/J.1399-3054.1986.TB02429.X

Sánchez, M. D., Mantell, C., Rodríguez, M., Martínez de la Ossa, E., Lubián, L. M., & Montero, O. (2005). Supercritical fluid extraction of carotenoids and chlorophyll *a* from *Nannochloropsis gaditana*. *Journal of Food Engineering*, *66*(2), 245-251. https://doi.org/10.1016/j.jfoodeng.2004.03.021

Saygıdeğer, S. (1996). *Lemna gibba L. ve Lemna minor L., (Lemnaceae)'nin morfolojik anatomik, ekolojik ve fizyolojik özellikleri*. *Ekoloji*, *5*(18), 8-11.

Saygıdeğer, S. (1997). *Seyhan Nehrinde bazı su bitkileri üzerine tarımsal kimyasalların etkileri* [The effects of agricultural chemicals on some aquatic plants in the Seyhan River]. *Hacettepe Fen ve Mühendislik Bilimleri Dergisi Seri A*, *18*, 35-43.

Saygıdeğer, S. D., Keser, G., & Dogan, M. (2013). Effects of lead on chlorophyll content, total nitrogen, and antioxidant enzyme activities in duckweed (*Lemna minor*). *International Journal of Agriculture and Biology*, *15*(1), 145-148.

Skillicorn, P., Spira, W., & Journey, W. (1993). *Duckweed aquaculture: A new aquatic farming system for developing countries.* World Bank.

Sokal, R. R., & Rohlf, F. J. (1995). *Biometry: The principles and practice of statistics in biological research*. W. H. Freeman and Co.

Sońta, M., Rekiel, A., & Batorska, M. (2019). Use of duckweed (*Lemna L.*) in sustainable livestock production and aquaculture – A review. *Annals of Animal Science*, *19*(2), 257-271. https://doi.org/10.2478/aoas-2018-0048

Topal, M., Karagözoğlu, B., Öbek, E., & Topal, I. (2011). Usage of some duckweeds in nutrient removal. *Mehmet Akif Ersoy University Journal of the Graduate School of Natural and Applied Sciences*, *2*(2), 12-28.

von Wirén, N., Gazzarrini, S., Gojon, A., & Frommer, W. B. (2000). The molecular physiology of ammonium uptake and retrieval. *Current Opinion in Plant Biology*, *3*(3), 254-261. https://doi.org/10.1016/S1369-5266(00)80074-6

Wanapat, M. (1994). Supplementation of straw-based diets for ruminants in Thailand. *Proceedings of Sustainable Animal Production and the Environment*. *The 7th AAAP Animal Science Congress*, Indonesia. pp. 25-38.

Wang, C., Zhang, S. H., Li, W., Wang, P. F., & Li, L. (2011). Nitric oxide supplementation alleviates ammonium toxicity in the submerged macrophyte *Hydrilla verticillata* (Lf) Royle. *Ecotoxicology and Environmental Safety*, *74*(1), 67-73.

Wang, W., Yang, C., Tang, X., Gu, X., Zhu, Q., Pan, K., Hu, Q., & Ma, D. (2014). Effects of high ammonium level on biomass accumulation of common duckweed *Lemna minor* L. *Environmental Science and Pollution Research*, *21*(24), 14202-14210. https://doi.org/10.1007/s11356-014-3353-2

Wersal, R. M., & Madsen, J. D. (2012). *Aquatic plants their uses and risks. A review of the global status of aquatic plants*. FAO, Rome. 97 p.

Wett, B., & Rauch, W. (2003). The role of inorganic carbon limitation in biological nitrogen removal of extremely ammonia concentrated wastewater. *Water Research*, *37*(5), 1100-1110. https://doi.org/10.1016/S0043-1354(02)00440-2

Whitehead, A. J., Lo, K. V., & Bulley, N. R. (1987). The effect of hydraulic retention time and duckweed cropping rate on nutrient removal from dairy barn wastewater. In K. R. Reddy and W. H. Smith (Eds.), *Aquatic Plants for Water Treatment and Resource Recovery* (pp. 697-703). Magnolia Publishing Inc.

Yılmaz, Z. (2004). *Nutrient removal from S.U. campus wastewater by duckweed (Lemna minor L.)* [M.Sc. Thesis. Selçuk University].

Zou, N., & Richmond, A. (2000). Light-path length and population density in photoacclimation of *Nannochloropsis* sp. *(Eustigmatophyceae)*. *Journal of Applied Phycology*, *12*, 349-354. https://doi.org/10.1023/A:1008151004317