JEE Journal of Ecological Engineering

Journal of Ecological Engineering, 2025, 26(2), 56–72
https://doi.org/10.12911/22998993/195971
ISSN 2299–8993, License CC-BY 4.0

Received: 2024.10.23
Accepted: 2024.12.15
Published: 2025.01.01

Influence of temperature and photoperiod on the growth and development of *Lemna trisulca* and *Lemna minor* in controlled conditions

Joanna Sender[1*], Antoni Grzywna[2]

[1] Department of Hydrobiology and Protection of Ecosystems, University of Life Sciences in Lublin, Dobrzańskiego 37, 20-262 Lublin, Poland
[2] Department of Environmental Engineering and Geodesy, University of Life Sciences in Lublin, Leszczyńskiego 7, 20-069 Lublin, Poland
* Corresponding author's e-mail: joanna.sender@up.lublin.pl

ABSTRACT

The aim of the study was to analyze the growth and development of two species of duckweed: *Lemna minor* and *Lemna trisulca* under different combinations of temperature (15–25 °C) and photoperiod (12–24 hours), while controlling physicochemical parameters such as electrical conductivity, pH, and oxygen content. The plants were placed in synthetic nitrogen medium, and their growth was monitored for 40 days. Statistical methods, including analysis of variance (ANOVA) and principal component analysis (PCA), were used for data analysis. The results showed that both species grow better at higher temperatures (25 °C) and longer photoperiods (24 hours). *Lemna minor* achieved higher surface coverage up to 58.4% and biomass over 1.44 g fresh weight under optimal conditions (25 °C and a 12-hour photoperiod), indicating its high efficiency in utilizing favourable conditions. *Lemna trisulca*, on the other hand, showed a more stable increase in biomass (1.03 g fresh weight) and cover 45.8% under its extreme conditions (15 °C and 12-hour photoperiod). Regarding adaptation to changing physicochemical conditions, *Lemna minor* responded better to favourable parameters, achieving higher growth rates under optimal pH 6.05 and electrical conductivity 31.6 µS/cm, while *Lemna trisulca* showed stable growth even in more variable conditions, with minimal decrease in growth under higher conductivity (583 µS/cm) and lower pH (6.96). The research findings reveal that *Lemna minor* is more competitive under optimal conditions, which may result from its more efficient use of available resources. Its rapid growth makes it particularly valuable in bioremediation, while *Lemna trisulca* may cope better with variable aquatic conditions. The conclusions emphasize the adaptive differences between the two species, which is significant for managing aquatic ecosystems. *Lemna minor* is suitable for stable environments, whereas *Lemna trisulca* finds applications in more variable conditions, indicating their diverse potential uses in environmental protection and bioremediation. These studies provide important data on the adaptive capabilities of duckweed, which is essential for effective management of aquatic ecosystems.

Keywords: aquatic ecosystem management, duckweed, bioremediation, light, temperature

INTRODUCTION

Lemnaceae are small aquatic plants commonly found in freshwater ecosystems worldwide [Landolt 1986, Lemon et al. 2001, Anderson and Martin 2005]. Among the most frequently encountered species are *Lemna minor* and *Lemna trisulca*, which play a key role in aquatic ecosystems. Duckweeds are some of the smallest angiosperms and are characterized by their rapid growth rate, making them a popular choice for ecological and biotechnological studies. Their ability to quickly accumulate biomass makes them an important component of primary production in water bodies, serving as a food source for many aquatic organisms, such as fish and invertebrates [Ziegler et al. 2015, Anderson et al. 2011, Tippery and Les 2020]. Despite their ecological importance, the effects of combined environmental factors on duckweed physiology remain underexplored, particularly in regard to the simultaneous influence of temperature and photoperiod.

As autotrophic plants, duckweeds not only produce oxygen through photosynthesis, improving water quality, but also play a crucial role in regulating light availability, which affects other photosynthetic organisms like algae. Their rapid growth rate makes them particularly sensitive to environmental changes, including temperature and photoperiod, which are significant factors influencing duckweed growth and competitive dynamics in freshwater ecosystems [Ceschin et al., 2020; Sun et al., 2020].

Temperature and light are the primary factors influencing duckweed physiology [Strzałek and Kufel 2021; Ziegler et al., 2015; Walsh et al., 2021; Lasfar et al., 2007]. Temperature regulates the rate of photosynthesis, metabolism, and cell division. The optimal growth temperature for duckweeds ranges from 15 °C to 30 °C [Ziegler et al. 2015], though tolerance for extreme temperatures can vary by species. For instance, *Lemna minor* can better tolerate higher temperatures compared to *Lemna trisulca*, which prefers more moderate conditions [Kufel et al., 2012; Lasfar et al., 2007]. Lower temperatures slow down metabolism, negatively impacting the plants' growth and development [Cui and Cheng, 2015].

Light, on the other hand, is a crucial factor necessary for photosynthesis, and its availability—both in terms of photoperiod and intensity—can significantly affect plant growth [Ceschin et al., 2020]. Photoperiod, or the length of daylight, regulates the growth cycles of many plants, including duckweeds. Increased light exposure can enhance photosynthetic intensity, thereby accelerating duckweed biomass growth. However, excessive light exposure can lead to photoinhibition—stress caused by light overexposure [Cui and Cheng, 2015]. While the effects of temperature and light have been studied independently, few studies investigate their combined impact, which could reveal important interactions affecting duckweed adaptation and growth.

Previous studies suggest that *Lemna minor* generally prefers warmer temperatures, with optimal conditions varying by cultivation and location [Ge et al., 2012]. In contrast, *Lemna trisulca* is less tolerant to environmental fluctuations, which can impact its growth and adaptability in diverse habitats [Landolt, 1986].

Despite numerous studies on the influence of temperature and photoperiod on the development of individual duckweed species, there are relatively few that analyze the simultaneous impact of these two factors. Most previous research has focused on single variables, often overlooking potential interactions between temperature and photoperiod that could significantly affect biomass growth and plant adaptation to changing environmental conditions. Understanding the synergistic effects of these factors is crucial for effective management of aquatic ecosystems and for biotechnological applications.

This study aims to fill a critical gap by investigating the combined effects of temperature and photoperiod on the growth of *Lemna trisulca* and *Lemna minor*. Unlike previous studies focusing on isolated variables, this research provides a novel, integrative perspective essential for understanding duckweed adaptation and potential applications in water quality management. Additionally, physicochemical parameters such as electrical conductivity, oxygen content, and pH were controlled to ensure comprehensive insight into environmental adaptability.

MATERIALS AND METHODS

The plant material was collected from ponds in the Ciemięga River Valley and then transported to the laboratory. Upon arrival, the plants were rinsed with tap water and placed in separate containers (one per species) filled with synthetic nitrogen medium following the formulation by Appenroth, Teller, and Horn [1996]. The species were then transferred to clear PET cups (100 mL, Ø 70 mm), which were submerged in trays filled with 2 L of nutrient medium. Each tray contained four cups with *Lemna minor* (10 fronds per cup) and four cups with *Lemna trisulca* (10 fronds per cup). The total number of cups across various cultivation setups was as follows: 4 replicates × 2 species × 4 growing conditions = 32 cups.

The study utilized a single level of light intensity, with an average photosynthetic photon flux density (PPFD) of 262 μmol m^{-2} s^{-1}, consistent with the optimal growth conditions for duckweed as reported in the literature. Two photoperiod conditions were applied: continuous light (24 h light: 0 h dark) and a balanced light-dark cycle (12 h light: 12 h dark). The light intensity was chosen based on the light saturation points for duckweed growth reported by Landolt and Kandeler [1987]. Cultivation was conducted in a pHcbi MLR-350/352 growth chamber, manufactured in 2022, designed for plant growth, which maintained stable temperature and light conditions according to programmed settings, while humidity was kept constant.

Experimental variants: A: fotoperiod_24h_temp_15 °C, B: fotoperiod_24h_temp_25 °C, C: fotoperiod_12h_temp_15 °C, D: fotoperiod_12h_temp_25 °C

Every three days, sample rotation was conducted by changing the positions of the cups within the growth chamber to ensure uniform exposure to light and other environmental conditions. Additionally, the following parameters were monitored and assessed: percentage coverage of each cup by fronds (%), determined using ImageJ software; frond count, mat width (cm, using a ruler), sediment amount (coverage % and thickness in cm), root length (cm, measured from the base of the frond), and fresh weight of selected fronds (g) to estimate biomass. Electrolytic conductivity, water pH, and water oxygen saturation were also analyzed using a Hanna HI 98194 multiparameter meter, with measurements taken weekly, continuing up to the 40th day of incubation. The nutrient medium remained unchanged throughout the experiment.

Once per week, fronds from each cup were photographed under standardized lighting conditions using a Nikon Z6 camera with a Z MC 105 mm f/2.8 VR S lens. The images were processed using CorelDRAW Photo Paint X5, and then ImageJ software was used to measure the frond surface area.

Additionally, the following parameters were monitored and assessed: percentage coverage of each cup by fronds (%), determined using ImageJ software; frond count; mat width (cm, measured with a ruler); sediment amount (coverage % and thickness in cm); root length (cm, measured from the base of the frond); and fresh weight of selected fronds (g) to estimate biomass.

Statistical analysis

A range of statistical methods was applied to analyze data from the experiments on Lemna minor and Lemna trisulca. To assess the impact of different experimental conditions, such as photoperiod and temperature, on morphological variables (surface coverage, biomass, root length), analysis of variance (ANOVA) was employed. Both one-way and three-way ANOVA were conducted, allowing for evaluation of interactions between variables and identification of their influence on the observed traits.

For data that did not meet the assumptions of normal distribution or homogeneity of variances, the Kruskal-Wallis test was used. To further analyze significant differences between groups, Tukey's post-hoc tests were performed, enabling detailed identification of pairs of conditions that differed significantly from one another.

To reduce data dimensionality and identify the main patterns among variables describing the growth and development of *Lemna minor* and *Lemna trisulca* under various environmental conditions, principal component analysis (PCA) was conducted.

Additionally, regression analysis, both linear and nonlinear, was used to assess the relationships between dependent and independent variables and to determine the line of best fit for selected variables.

To analyze the relationships between variables, correlation matrices were calculated using Pearson's correlation coefficient. This method is suitable for normally distributed data and measures the linear relationship between variables. For each pair of variables, a correlation coefficient value was calculated: values close to +1 indicate a strong positive linear relationship, values close to -1 indicate a strong negative linear relationship, and values near 0 suggest no linear relationship.

The correlation matrix was visualized using colored squares with the corrplot() function from the R package. The color of the squares corresponded to the strength and direction of the correlation: red represented negative correlations, while blue indicated positive correlations. The plots displayed only the upper half of the correlation matrix (without repetitions), allowing for a clearer presentation of the results.

All statistical analyses were conducted using R Studio and Excel, with a significance level set at $\alpha = 0.05$. The results were presented in the form of charts and tables, allowing for a detailed comparison of the effects of the experimental conditions on the variables studied.

RESULTS

Under condition A (24h photoperiod, 15 °C temperature), the average surface coverage of *Lemna minor* was 19.17%, while in condition D (12h photoperiod, 25 °C temperature), it reached 58.41%. The average thickness of the plants increased from 0.51 cm in condition A to 2.01 cm in condition D. Sediment coverage was 51.35% in condition B, with sediment thickness varying between 0.06 cm and 0.70 cm depending on the conditions.

Electrical conductivity values reached a maximum of 154.65 $\mu S \cdot l^{-1}$ under condition C, with pH values ranging from 5.12 to 6.88. The average oxygen content was 8.64 mg/l in Condition A, while frond counts ranged from 1.38 to 3.02. Root length varied from an average of 0.44 cm in condition A to 1.17 cm in condition C, and fresh biomass ranged from 0.43 g to 1.45 g. Detailed values are presented in Table 1.

For *Lemna trisulca*, the average surface coverage was 17.75% in condition A, reaching 45.76% in condition C. Plant thickness ranged from 0.84 cm in condition B to 1.36 cm in condition C. Sediment coverage was 44.52% in condition B, with sediment thickness ranging from 0.13 cm to 0.57 cm.

The maximum electrical conductivity for *Lemna trisulca* was recorded under condition C, at 583.29 $\mu S \cdot l^{-1}$, with pH values between 6.65 and 7.54. The average oxygen content was 8.08 mg/l in condition B, with frond counts varying from 2.87 to 6.82. Root length averaged from 0.54 cm in condition B to 0.60 cm in condition C, and fresh biomass ranged from 0.43 g to 1.45 g (Table 1).

Lemna minor vs. Lemna trisulca under different cultivation conditions

Coverage

Under condition A (24h photoperiod, 15 °C temperature), both species exhibit low coverage values, with *Lemna minor* performing better than *Lemna trisulca*. This may suggest that *Lemna minor* is more efficient in utilizing a longer photoperiod, even at lower temperatures. Increasing the temperature to 25°C while maintaining the same photoperiod (condition B) leads to further increases in plant coverage, with *Lemna minor* again dominating. Values for *Lemna trisulca* also rise but do not reach the levels seen in *Lemna minor*.

Introducing a shorter photoperiod (12 hours) at the same temperature of 15 °C (condition C) results in a significant increase in plant coverage for both species; however, *Lemna minor* continues to show superior results. In condition D (12h photoperiod, 25 °C temperature), both the increased temperature and shorter photoperiod yield the highest coverage values for *Lemna minor*, suggesting that this combination of conditions is optimal for this species. *Lemna trisulca* also improves its performance but remains significantly behind *Lemna minor* (Fig. 1).

An analysis of variance (ANOVA) was conducted to assess the impact of the different conditions (A, B, C, D) on the coverage of *Lemna minor* and *Lemna trisulca*. The results revealed significant differences in the average coverage values across the various conditions ($F(3, 24) = 7.692$, $p < 0.001$). The average coverage for each condition was as follows: condition A: 19.17% (SD = 6.53), condition B: 9.46% (SD = 3.93), condition C: 34.88% (SD = 24.60), and condition D: 58.41% (SD = 29.73).

A Tukey post-hoc test comparing the average plant coverage across different conditions (A, B, C, D) indicated significant differences between the groups. The largest difference in coverage was observed between condition D and condition B, with condition D showing 32.54% greater coverage compared to condition B. Condition C exhibited 24.22% higher coverage than condition B, while condition D had 21.01% higher coverage than condition A. Other differences, though smaller, were also statistically significant. Condition C demonstrated a 12.68% higher coverage compared to condition A, and condition D was 8.32% higher than condition C. An exception was noted for the negative difference between conditions B and A, where the coverage was 11.53% lower in condition B compared to condition A (Fig. 2).

The results of the Tukey test indicate that the conditions under which the plants were studied have a significant impact on their coverage, with all comparisons being statistically significant. Conditions C and D show significantly higher coverage values compared to conditions A and B.

Biomass

Lemna minor reached the highest biomass under condition B, with average values around 1.5 g, suggesting that a long photoperiod and higher temperature promote its growth. In contrast, under conditions A and C, where the temperature is lower, the biomass of *Lemna minor* averaged 0.8 g and 0.6 g, respectively, which may indicate this species' sensitivity to low temperatures. *Lemna trisulca* showed a varied response to the same conditions. Under condition B, the biomass of this species averaged 1.0 g, suggesting that it too may benefit from a long photoperiod and higher temperature, although its growth is lower

Table 1. Comparison of selected traits of *Lemna minor* and *Lemna trisulca* and environmental conditions in different conditions (A, B, C, D)

Condition		Lemna minor				Lemna trisulca			
		A	B	C	D	A	B	C	D
Lemna cover (%)	Mean	19.166	9.459	34.87	58.414	31.80	17.748	45.76	18.50
	SD	6.525	3.931	24.59	29.72	11.63	8.68	17.50	3.855
	Median	20	10	20	50	30	15	50	20
	Min	0	0	15	8	15	10	20	10
	Max	30	15	90	100	50	35	75	25
Lemna thicknes (cm)	Mean	0.5100	0.3310	1.0966	2.0120	1.0322	0.8358	1.3616	0.5132
	SD	0.19	0.14	0.81	1.52	0.28	0.37	0.57	0.08
	Median	0.5	0.3	45413	45292	45292	0.9	45352	0.5
	Min	0.0	0.0	0.1	0.3	0.6	0.3	0.6	0.3
	Max	0.7	0.5	45353	45417	45413	45413	45353	0.7
Sediment cover (%)	Mean	9.958	51.351	39.371	9.565	44.51	77.09	60.28	52.31
	SD	19.73	50.32	33.40	11.86	47.55	42.18	26.72	45.21
	Median	0	100	25	0	15	100	70	60
	Min	0	0	0	0	0	0	10	0
	Max	50	100	95	35	100	100	90	100
Sediment thicknes (cm)	Mean	0.341	0.063	0.699	0.116	0.90	0.12	0.57	0.28
	SD	0.655	0.086	0.922	0.127	0.733	0.084	0.177	0.476
	Median	0.0	0.1	0.1	0.0	43831	0.15	0.60	0.10
	Min	0	0	0	0	0.0	0.0	0.3	0.0
	Max	45474	0.5	45414	0.3	45474	0.3	1.0	45352
Conductivity (u/S)	Mean	32.59	38.09	154.65	31.62	37.80	40.44	583.29	41.83
	SD	3.926	5.697	113.695	14.940	10.724	9.014	655.465	8.895
	Median	33.88	36.60	152.40	34.99	37.8	41.5	364.1	41.9
	Min	15.36	45465	36.90	41730	18.24	45589	39.90	45530
	Max	35.11	46.80	332.50	53.50	58.2	64.8	2164.0	56.6
pH	Mean	5.121	5.740	6.875	6.053	7.538	6.649	6.964	7.501
	SD	0.7410	0.3488	1.1825	1.2509	1.5373	0.9376	0.9516	0.9685
	Median	24198	28246	17349	45357	454	425	319	111
	Min	194	454	456	464	468	355	129	455
	Max	433	209	133	246	19603	11567	46600	18507
Oxygen content (mg/l)	Mean	8.640	8.100	96.478	8.697	8.994	8.085	102.909	8.576
	SD	0.2670	0.4561	5.2141	0.5711	0.6126	0.7867	15.5293	1.2172
	Median	20302	46600	96.60	27973	32721	31959	103.40	34912
	Min	15919	12936	88.80	21002	43313	45108	77.60	36281
	Max	33482	14093	107.40	45301	43374	45545	130.20	45575
Frond number	Mean	1.69	1.37	3.01	1.91	4.61	2.87	6.82	2.99
	SD	0.65	0.73	1.21	1.20	2.07	1.05	2.63	0.57
	Median	2	1	3	2	3	3	6	3
	Min	0	0	1	1	2	1	1	1
	Max	3	3	6	22	12	6	15	5
Root length (cm)	Mean	0.43	0.41	1.17	1.18	0.69	0.53	0.59	0.36
	SD	0.31	0.28	0.72	1.00	0.54	0.64	0.37	0.22
	Median	0.30	0.40	1.20	0.81	0.6	0.4	0.5	0.3
	Min	0.0	0.0	0.1	0.1	0.1	0.1	0.1	0.1
	Max	452	454	455	454	3.0	7.0	452	0.9
Lemna biomass (g)	Mean	0.73	0.42	0.88	1.44	0.98	0.88	1.03	0.95
	SD	0.33	0.25	0.13	0.33	0.07	0.20	0.14	0.02
	Median	0.85	0.50	0.90	18264	0.95	0.95	0.95	0.95
	Min	0.00	0.00	0.65	0.50	0.950	0.095	0.950	0.950
	Max	1.1	0.75	1.1	2	1.04	1.52	1.14	1.14

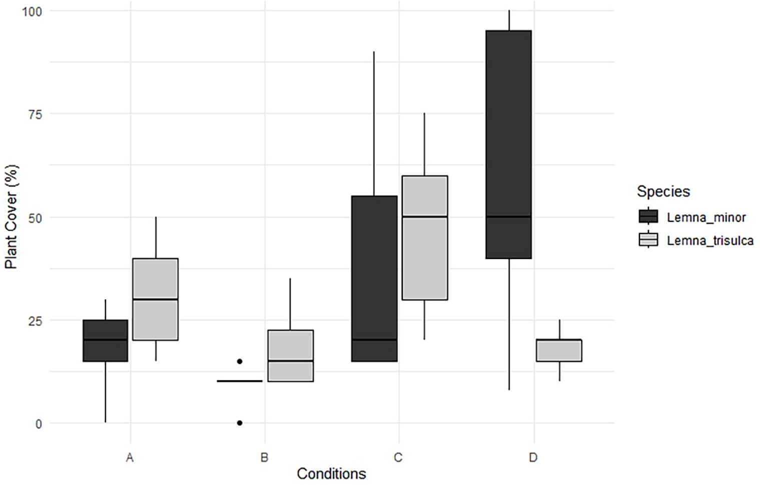

Figure 1. Plant cover comparison between *Lemna minor* and *Lemna trisulca*

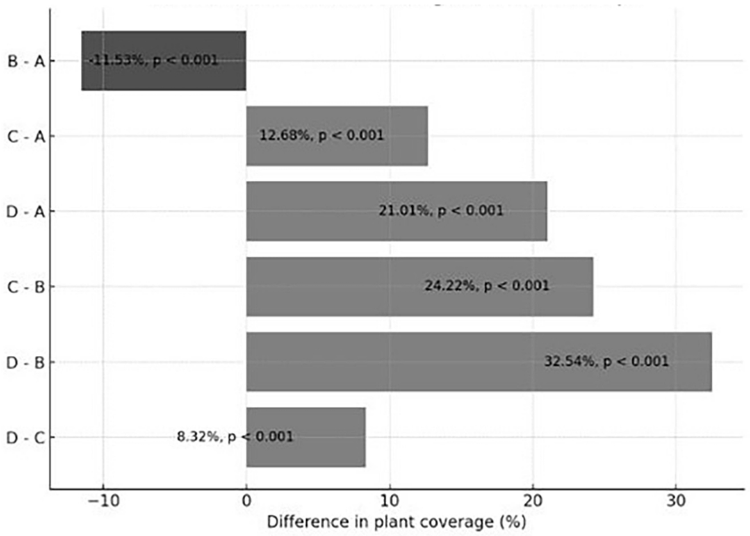

Figure 2. Tukey test: plant coverage comparison across conditions

compared to *Lemna minor*. In conditions A and C, the biomass of *Lemna trisulca* averaged 0.5 g and 0.4 g, respectively, highlighting that this species is more sensitive to lower temperatures and shorter light exposure. In condition D, where the temperature is higher (25 °C) but the photoperiod is shorter (12 h), *Lemna trisulca* exhibited an average biomass of 0.7 g, suggesting that this species may respond well to higher temperatures but requires a longer photoperiod (Fig. 3).

The analysis of variance (ANOVA) revealed a significant difference in biomass between the studied species, suggesting that species has a significant impact on this parameter. The F-value for species is 102.1 ($p < 2e\text{-}16$), confirming that the differences are statistically significant.

The cultivation conditions also had a significant effect on biomass. The F-value for conditions is 397.4 ($p < 2e\text{-}16$). This indicates that changes in factors such as temperature and photoperiod have a substantial impact on plant biomass development. Additionally, the analysis revealed a significant interaction between species and cultivation conditions. The F-value for this interaction is 255.9 ($p < 2e\text{-}16$). This implies that the effect of conditions on biomass varies depending on the species. The summary of the Tukey test results reveals significant differences in plant biomass across various cultivation conditions. The mean square error was 0.0860 (df 1350). The average biomass across all analysed conditions was 1.0424 g, with a coefficient of variation (CV) of

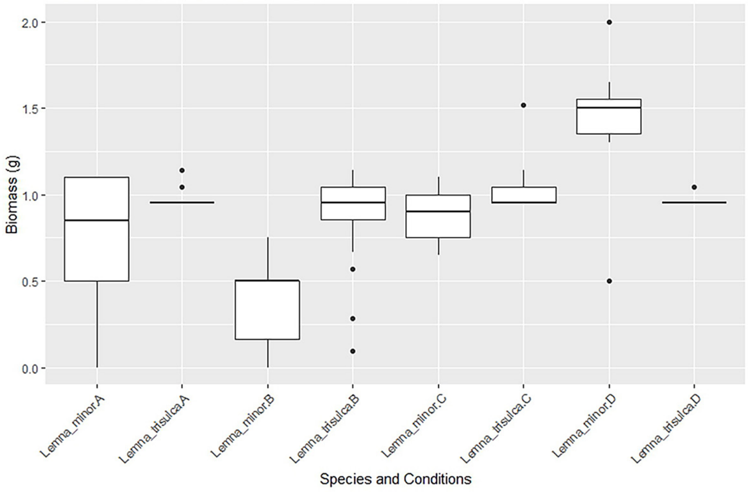

Figure 3. Biomass depending on species and photoperiod and temperature conditions

28.14%. The Tukey test was conducted for four conditions (A, B, C, and D), each representing different cultivation conditions. The calculated standardized range was 3.6377, with a significance level (alpha) of 0.05. Analysing the mean biomass in each condition showed that condition D, with a 12-hour photoperiod at 25 °C, had the highest average biomass at 1.3105, suggesting that plants in this condition performed best.

In contrast, condition A, which had a 24-hour photoperiod at 15 °C, yielded an average biomass of 0.8757, while condition C, with a 12-hour photoperiod at 15 °C, achieved 0.9408. The lowest mean biomass, 0.7221, was observed under condition B, characterized by a 24-hour photoperiod at 25 °C. This condition was grouped with condition D, indicating a significant difference compared to conditions A, C, and D. Conditions A and C fell between the extremes, with condition A showing a significant difference from condition B, but not from condition C. The Tukey test results clearly highlight the dominance of condition D, which was the leading condition for biomass. Conversely, condition B, with the lowest biomass value, demonstrated significantly poorer results in the same category.

Thickness of the surface layer of Lemna

Lemna minor and *Lemna trisulca* exhibited differences in the thickness of their surface layer depending on photoperiod and temperature. *Lemna trisulca* appeared to be more sensitive to changes in light conditions, while *Lemna minor* tolerated higher temperatures better, even with shorter light exposure. These findings suggested that the two species employed distinct adaptive strategies. (Fig. 4).

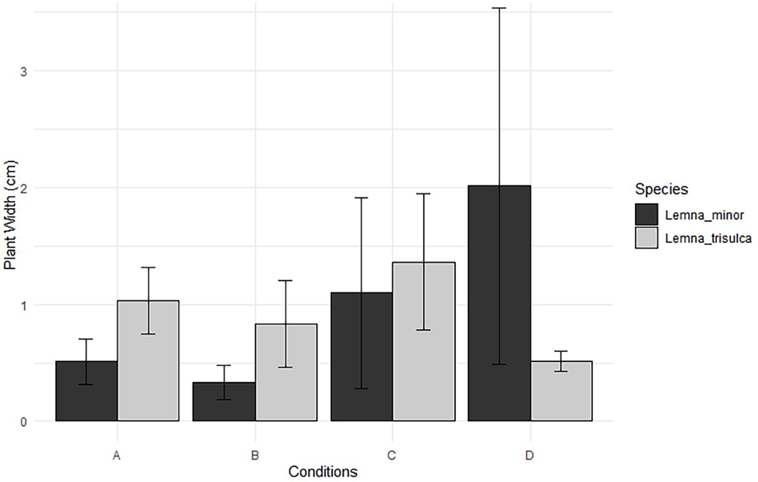

Figure 4. Thickness of Lemna species layer depending on species and photoperiod and temperature conditions

Under conditions with a long photoperiod (24 hours) and low temperature (15 °C), *Lemna minor* showed moderate growth in width, suggesting that it could adapt well to such conditions. During the same period, *Lemna trisulca* exhibited slightly greater width, indicating better adaptation to a long photoperiod in a cooler environment.

When conditions shifted to a longer photoperiod and higher temperature (25 °C), *Lemna minor* began to reach larger widths, indicating a positive influence of these conditions on its development. Meanwhile, *Lemna trisulca* reached even larger sizes, suggesting a preference for warmer conditions combined with extended light exposure.

In conditions with a shorter photoperiod (12 hours) and lower temperature (15 °C), the widths of both species were similar, with a slight advantage for *Lemna trisulca*. The shorter light exposure may have limited growth, but the moderate conditions appeared to support both species. Under the shorter photoperiod (12 hours) and higher temperature (25 °C), *Lemna minor* showed a noticeable increase in width, suggesting its ability to adapt to warmer surroundings even with reduced light duration. In contrast, the width of *Lemna trisulca* was smaller under these conditions (Fig. 4).

Frond number

Lemna minor demonstrated a tendency for more intensive growth in the number of fronds under conditions with a shorter photoperiod, which suggested that it could better adapt to moderate temperatures. In contrast, *Lemna trisulca* exhibited a greater number of fronds under longer photoperiods and higher temperatures, indicating its preferences for growth conditions (Fig. 5).

In condition A, where the photoperiod was 24 hours at a temperature of 15 °C, both *Lemna minor* and *Lemna trisulca* achieved a low number of fronds. This suggested that prolonged light exposure combined with lower temperatures did not favor the intensive development of either species, possibly due to their limited reproductive capacity under such conditions.

When conditions shifted to a longer photoperiod and higher temperature (25 °C), a significant increase in the number of fronds was observed, particularly in *Lemna trisulca*, which became more dominant compared to *Lemna minor*. This indicated that warmer conditions and extended light exposure facilitated intensive growth and reproduction of this species. Under condition C, with a shorter photoperiod (12 hours) and a temperature of 15 °C, both plants attained a considerably higher number of fronds, with *Lemna minor* appearing to gain a particular advantage.

In condition D, where the photoperiod remained short (12 hours) but the temperature increased to 25 °C, both *Lemna minor* and *Lemna trisulca* exhibited a lower number of fronds compared to condition C (Fig. 5).

Root Length

Lemna minor generally demonstrated greater root lengths than *Lemna trisulca* across both temperature settings and photoperiods. Specifically, at 15 °C, both species exhibited a wider range of root lengths under the 12-hour photoperiod compared to the 24-hour photoperiod. In this condition, *Lemna minor* showed a higher median root length than *Lemna trisulca*, along with several outliers, indicating that some individuals had

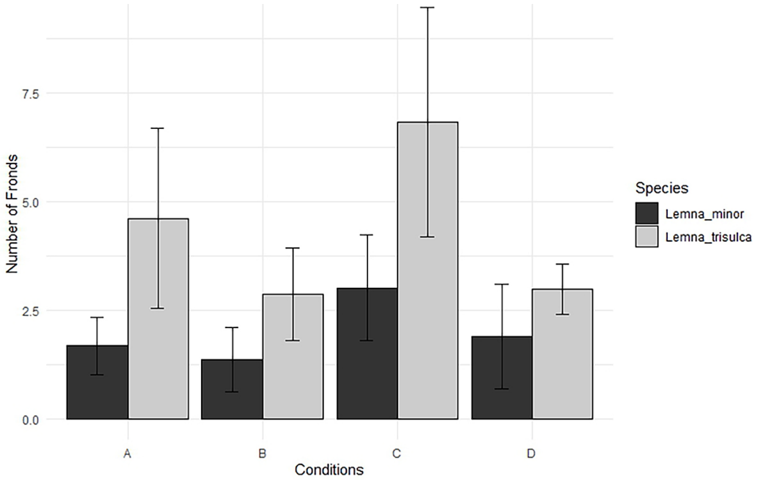

Figure 5. Frond number of Lemna species depending on species and photoperiod and temperature conditions

significantly longer roots. At 25 °C, root lengths for both species were noticeably shorter, particularly for *Lemna minor* during the 24-hour photoperiod. This observation suggested that prolonged light exposure at elevated temperatures could adversely affect root development. Conversely, *Lemna trisulca* consistently exhibited shorter root lengths compared to *Lemna minor* in both photoperiods. Furthermore, the 12-hour photoperiod typically resulted in longer root lengths for both species when compared to the 24-hour photoperiod at the same temperature. This finding implied that both species may experience stress or reduced growth potential under extended light exposure in warmer conditions. The presence of outliers, particularly in *Lemna minor*, indicated significant variation in growth responses within the species, suggesting that some individuals thrived under the given conditions (Fig. 6).

Sediment coverage analysis

Both *Lemna* species achieved higher sediment coverage values under the 12-hour photoperiod conditions. *Lemna minor* exhibited a median coverage of approximately 50%, which was significantly higher than that of *Lemna trisulca*, which had a median coverage of around 35%. Additionally, *Lemna minor* demonstrated greater variability in coverage, reaching up to 100%.

In contrast, the longer light exposure associated with the 24-hour photoperiod appeared to negatively impact the production of sediment in both species. This trend indicated that extended photoperiods might hinder the ability of both *Lemna minor* and *Lemna trisulca* to produce sediment effectively (Fig. 7).

Lemna trisulca demonstrated a greater sediment thickness, particularly under the 24-hour

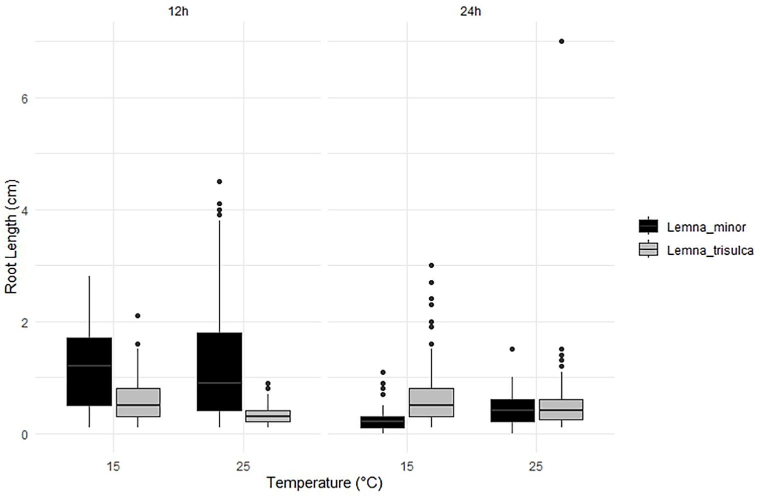

Figure 6. Root length of Lemna species depending on species and photoperiod and temperature conditions

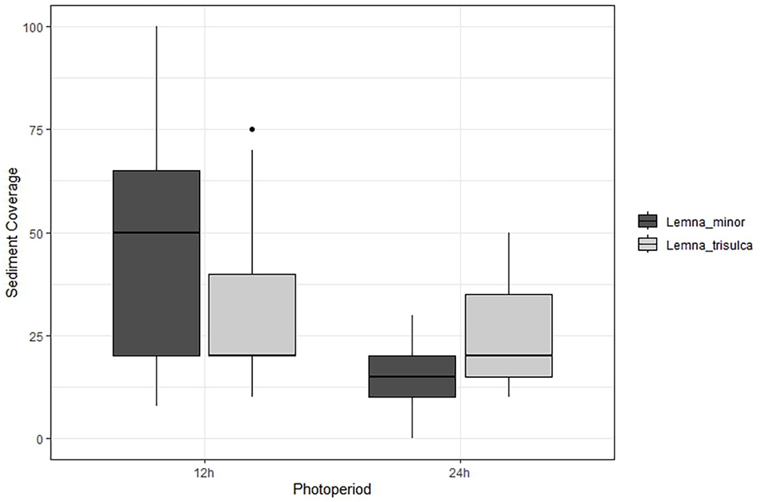

Figure 7. Sediment coverage of Lemna species depending on species and photoperiod conditions

photoperiod, where it reached a thickness of 2.5 cm. This finding indicated its higher susceptibility to sediment accumulation with prolonged light exposure. In contrast, the influence of photoperiod on *Lemna minor* was minimal, as its sediment thickness remained low, fluctuating between 0.2 to 0.8 cm (Fig. 8).

Analysis of morphological and environmental traits

In order to investigate morphological and environmental traits of the two aquatic plant species, *Lemna minor* and *Lemna trisulca*, a principal component analysis (PCA) was conducted. PCA allows for the reduction of multidimensional data into a few principal dimensions (components) that explain the majority of the variability within the data. The PCA results accounted for 59.9% of the total variance through the first two principal components.

The first component (Dim.1) explained 32.9% of the variance, while the second component (Dim.2) accounted for 27%. In the first dimension, the variables with the highest loadings were: coverage (%) (0.899) and plant thickness (cm) (0.851), suggesting that Dim.1 can be interpreted as a dimension primarily related to morphological traits that reflect the developmental status of both plant species. Additionally, variables such as the number of fronds (0.797) and oxygen concentration (mg/l) (0.729) also displayed high loadings. This may indicate that greater coverage and a higher number of fronds are associated with better oxygenation in the cultivation environment. When comparing *Lemna minor* and *Lemna trisulca*, it can be observed that *Lemna minor* (characterized by higher values on Dim.1) exhibited greater coverage and plant thickness, which could be a result of its more intense growth compared to *Lemna trisulca* under the same conditions.

The second component (Dim.2) was dominated by variables such as pH (0.787) and sediment thickness (cm) (0.637), with sediment coverage (%) (0.487) also significantly influencing this component. The high loading of the pH variable suggests that differences in the chemical environment (e.g., water acidity) play a crucial role in separating samples along this dimension. Higher values for *Lemna trisulca* indicate that this species may perform better in environments with higher pH and produces greater sediment layers.

Dim.3 (13.1% variance) was strongly associated with root length (cm) (0.907). This indicates that root length is a key variable for this component, and differences in this variable may contribute to differing nutrient uptake strategies between species.

Finally, Dim.4 (8.4% variance) was dominated by biomass (g) (0.725), suggesting that Dim.4 is related to overall biomass production efficiency, which is significant for both species under various cultivation conditions (Fig. 9).

The conducted principal component analysis (PCA) indicates that both species, *Lemna minor* and *Lemna trisulca*, primarily differ in terms

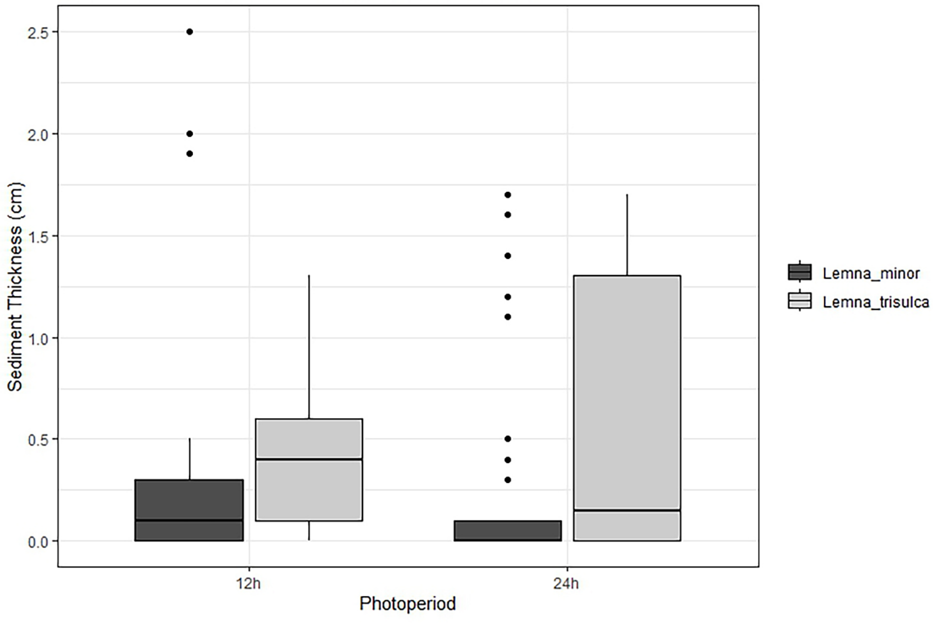

Figure 8. Sediment thickness of Lemna species depending on species and photoperiod conditions

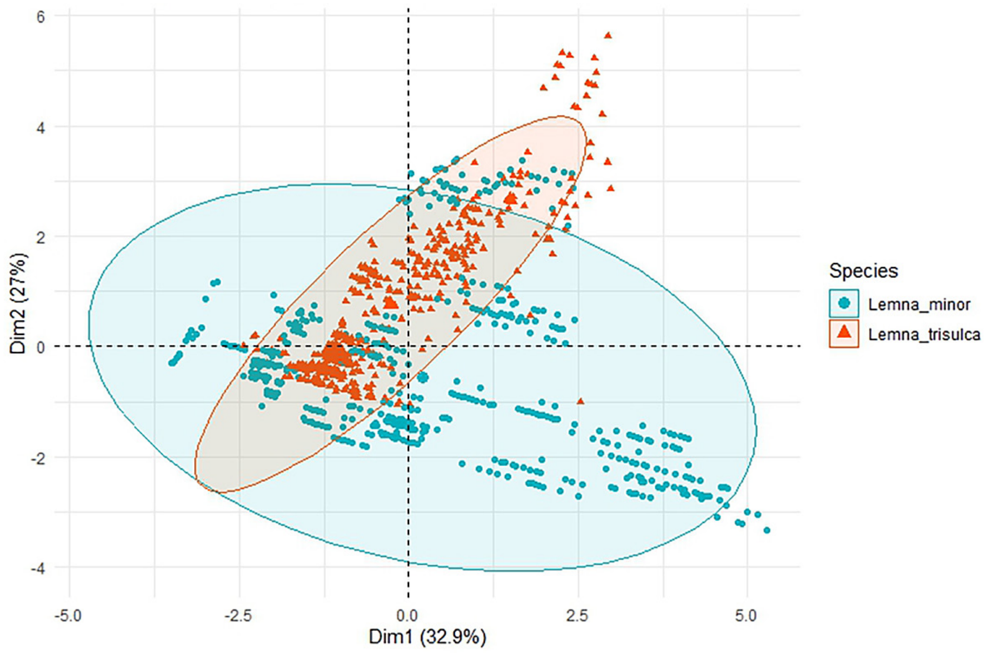

Figure 9. Principal component analysis (PCA) for studied Lemna species

of morphological traits (coverage, plant thickness, frond count) and physicochemical environmental conditions (pH, sediment thickness). *Lemna minor* is characterized by greater plant coverage and oxygenation in cultivation conditions, while *Lemna trisulca* appears to be better adapted to environments with higher pH and greater sediment accumulation.

Analysis of the impact of cultivation conditions on *Lemna minor*

The conducted analysis of variance (ANOVA) confirmed that cultivation conditions significantly influenced the thickness of the *Lemna minor* plant layer. The calculated F-value was 86.28 (F(3, 788) = 86.28, p < 2e-16), highlighting a strong statistical significance. The post-hoc Tukey test indicated that condition C (1.51 cm) significantly differed from conditions A (1.33 cm), B (1.14 cm), and D (0.01 cm). Additionally, condition A (1.33 cm) also significantly differed from condition D (0.01 cm), while condition B (1.14 cm) showed a significant difference compared to condition D (0.01 cm).

The ANOVA analysis for surface coverage also revealed a significant influence of conditions on the ability of *Lemna minor* to change in coverage. The variability in coverage was statistically significant, achieving an F-value of 141.8 (F(3, 788) = 141.8, p < 2e-16). Condition D (51.03%) significantly differed from conditions A (31.21%), B (21.12%), and C (35.71%). Conditions A (31.21%) showed a significant difference compared to condition C (35.71%) and B (21.12%), while condition B (21.12%) also significantly differed from condition C (35.71%).

The results of the variance analysis for sediment thickness indicate a significant impact of the conditions on this variable. The F-value was 56.88 (F(3, 788) = 56.88, p < 2e-16). The post-hoc Tukey test revealed that condition B (0.18 cm) significantly differed from condition A (0.40 cm), and condition C (0.57 cm) also significantly differed from condition A (0.40 cm). Furthermore, condition A (0.40 cm) significantly differed from condition D (0.22 cm).

The number of fronds of *Lemna minor* significantly varied depending on the cultivation conditions, achieving an F-value of 67.61 (F(3, 788) = 67.61, p < 2e-16). The post-hoc Tukey test indicated that condition C (4.23) significantly differed from conditions A (3.43), B (2.67), and D (3.01). Condition A (3.43) significantly differed from condition D (3.01), while condition B (2.67) also significantly differed from condition D (3.01).

Similarly, the root lengths of *Lemna minor* were statistically significantly varied, achieving an F-value of 44.15 (F(3, 788) = 44.15, p < 2e-16). Conditions C (1.45 cm) significantly differed from conditions A (0.59 cm) and D (0.52 cm), while condition A (0.59 cm) did not significantly differ from condition D (0.52 cm).

Additionally, biomass values significantly differed across the applied cultivation conditions, achieving an F-value of 429.9 (F(3, 788) = 429.9, p < 2e-16). Differences between groups indicated that condition C (1.45 g) significantly differed from conditions A (0.79 g), B (0.75 g), and D (0.87 g). Condition A (0.79 g) significantly differed from condition B (0.75 g), while condition D (0.87 g) did not significantly differ from condition A (0.79 g).

Analysis of the impact of cultivation conditions on *Lemna trisulca*

The analysis of variance (ANOVA) for the thickness of *Lemna trisulca* indicated a significant impact of cultivation conditions, achieving an F-value of 132.2 (F(3, 558) = 132.2, p < 0.001). The post-hoc Tukey test revealed significant differences among groups, where condition C (1.36 cm) differed significantly from conditions A (1.03 cm), B (0.84 cm), and D (0.51 cm). Additionally, condition A (1.03 cm) showed significant differences compared to conditions B (0.84 cm) and D (0.51 cm), while condition B (0.84 cm) significantly differed from condition D (0.51 cm).

Similarly, the ANOVA for percentage cover revealed a significant effect of cultivation conditions, with an F-value of 181.2 (F(3, 558) = 181.2, p < 0.001). The differences between groups indicated that condition C (45.76%) significantly differed from conditions A (31.81%), B (17.75%), and D (18.51%). Condition A (31.81%) also showed a significant difference when compared to condition D (18.51%) and B (17.75%), while condition B (17.75%) significantly differed from condition D (18.51%).

The analysis of sediment thickness also demonstrated a significant influence of cultivation conditions (F(3, 558) = 77.51, p < 0.001). The Tukey test indicated that condition A (0.90 cm) differed significantly from conditions C (0.57 cm), D (0.29 cm), and B (0.13 cm). Condition C (0.57 cm) also showed a significant difference compared to conditions D (0.29 cm) and B (0.13 cm).

In terms of frond number, the ANOVA confirmed a significant effect of cultivation conditions (F(3, 558) = 144.6, p < 0.001). The differences between groups revealed that Condition C (6.82) significantly differed from conditions A (4.61), B (2.87), and D (2.99). Condition A (4.61) also exhibited significant differences when compared to conditions B (2.87) and D (2.99).

Regarding root length of *Lemna trisulca*, the ANOVA demonstrated a significant effect of cultivation conditions (F(3, 515) = 14.65, p < 0.001). The post-hoc Tukey test indicated that condition A (0.68 cm) differed significantly from conditions B (0.52 cm) and D (0.31 cm). Condition C (0.59 cm) did not significantly differ from condition A (0.68 cm) but showed a significant difference compared to conditions B (0.52 cm) and D (0.31 cm).

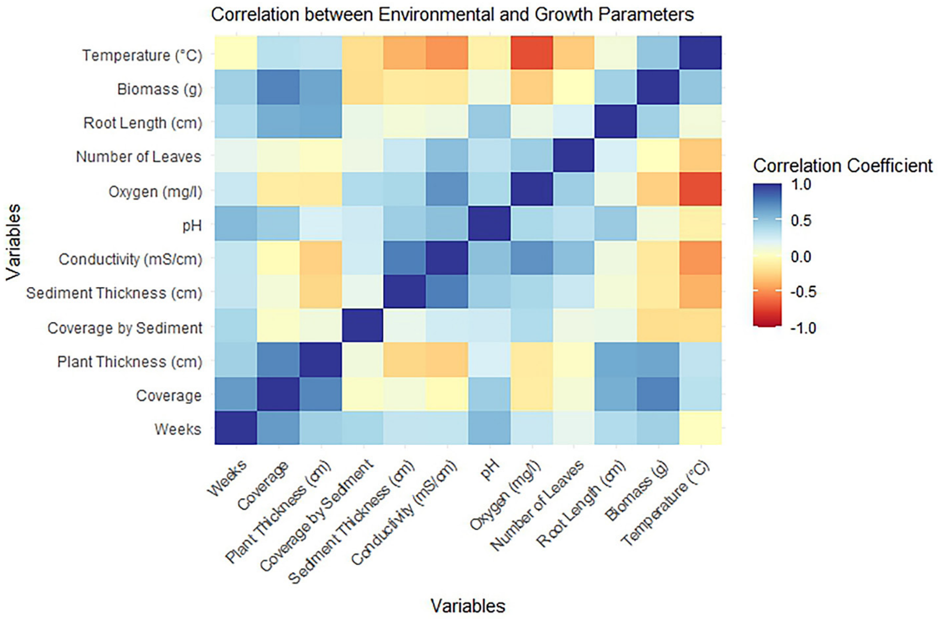

Figure 10. Correlation matrix for *Lemna minor*

Lastly, the biomass analysis also revealed a significant effect of cultivation conditions (F(3, 558) = 30.09, $p < 0.001$). The Tukey post-hoc test indicated that condition C (1.03 g) significantly differed from condition B (0.89 g). Conditions A (0.99 g) and D (0.96 g) did not significantly differ from condition C but showed differences from condition B.

Analysis of correlations in *Lemna minor* and *Lemna trisulca*

A strong positive correlation was observed for the species *Lemna minor* between the number of fronds and biomass. An increase in the oxygen content in the environment promotes an increase in the number of fronds in *Lemna minor*, while a higher temperature leads to an increase in the biomass of the plant. A strong negative correlation was found between the amount of sediment produced and the oxygen content. The greater the coverage by sediment, the likely lower the oxygen content in the water (Fig. 10).

In the case of *Lemna trisulca*, strong positive correlations (dark blue) were observed between the amount of sediment and its thickness (above 0.5), as well as between the number of fronds and root length. Strong negative correlations (dark red) were observed between temperature and the thickness of the duckweed cover and sediment coverage. Furthermore, biomass had a negative correlation with the amount of sediment, which may indicate that greater biomass is associated with lower sediment coverage (Fig. 11).

DISCUSSION

The results of the research on the influence of different environmental conditions on the development of *Lemna minor* and *Lemna trisulca* provide valuable insights into their adaptive capabilities in changing environmental parameters. The analysis of the effects of photoperiod and temperature on plant coverage, the thickness of the formed layer, the number of fronds produced, root length, and biomass, as well as the sediment created by these plants, reveals significant differences both between these species and within various experimental conditions.

Lemna minor achieves optimal growth at moderate temperatures (around 25 °C) and shows a decrease in growth at both low (6 °C) and high temperatures (35 °C), which is confirmed by the research of Van Dyck et al. [2021]. Increasing temperature also contributes to an increase in plant biomass, which is supported by earlier studies indicating a positive impact of higher temperatures on photosynthetic activity and growth efficiency [Paolacci et al., 2018; Pasos-Panqueva et al., 2024; Sender and Różańska-Boczula, 2024]. The increased biomass production, especially in nutrient-rich environments, can enhance bioremediation potential Studies on algae show

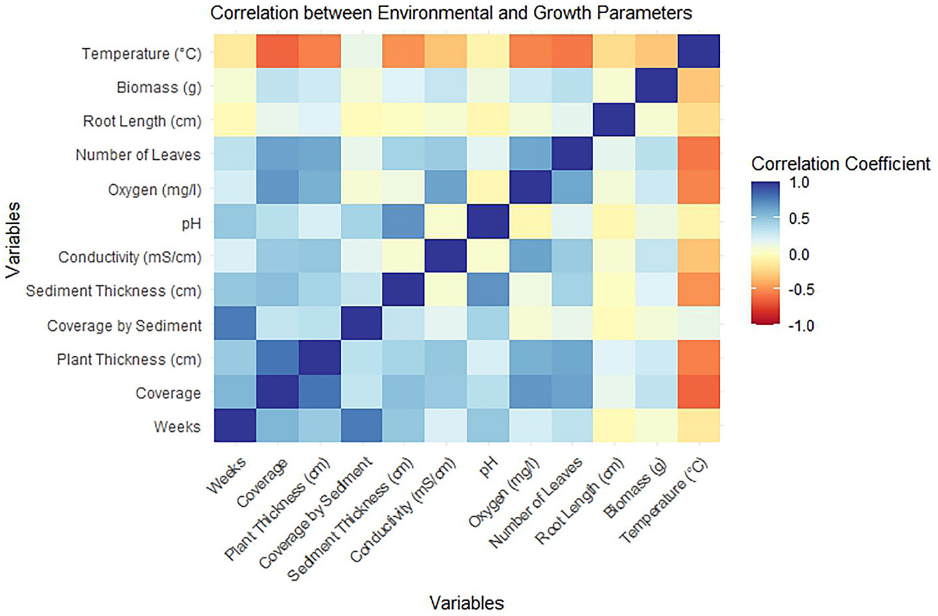

Figure 11. Correlation matrix for *Lemna trisulca*

that nutrient-rich wastewater can enhance growth rates and biomass yield [Zrimec et al., 2022; Liu et al., 2021; Puiseux-Dao, 2018]. Similarly, Lemna minor can thrive in nutrient-rich environments, often found in wastewater. Additionally, Lemna minor demonstrates an ability to adapt to a wide range of light durations, with higher light intensity (up to 262 μmol m^{-2} s^{-1}) promoting growth, and a longer photoperiod (14 hours) facilitating better development, underscoring the importance of light in their developmental cycle [Van Dyck et al. 2021].

In response to environmental stressors such as UV radiation and low temperatures, Lemna gibba accumulates flavonoids that provide protection against light and enhance photosynthetic efficiency [Akhtar et al., 2010]. Similarly, Lemna minor and other species can modify their photosynthetic apparatus and accumulate protective chemical compounds in response to a combination of light and temperature stress. The ability of these plants to survive and grow intensively under favorable conditions (temperature and light availability) could directly support bioremediation applications, as intense biomass growth in such conditions facilitates the uptake and accumulation of contaminants (Toyama et al. 2018). A strong positive correlation between the number of fronds and biomass in Lemna minor suggests that an increased availability of oxygen in the environment promotes frond growth, leading to improved photosynthetic conditions.

Furthermore, the use of wastewater not only supplies essential nutrients but also decreases the reliance on freshwater resources, making the process more sustainable [Liu et al., 2021; Magalhães et al., 2021; Velichkova et al., 2018]. Duckweed can thrive in these conditions, thereby enhancing biomass production while contributing to water remediation efforts.

The increased plant coverage and biomass efficiency of *Lemna minor* under higher temperatures and longer photoperiods underscore its potential application in bioremediation. This intensified growth under optimal conditions supports the removal of nutrients and contaminants from water. While findings by Appenroth et al. [2010] show that high density may eventually limit growth rates, rapid biomass accumulation in favorable conditions suggests that *Lemna minor* can efficiently contribute to nutrient removal before density limitations become restrictive. Similarly, Driever et al. [2005] observed that excessive density may reduce growth rates, which can impact the plant's adaptive capacity.

The analysis indicates that *Lemna minor* achieves higher plant coverage in environments with elevated temperatures and longer photoperiods, which further supports its suitability for bioremediation in such conditions. In contrast, in *Lemna trisulca*, strong positive correlations were observed between sediment quantity and its thickness, as well as between the number of fronds and root length. These results suggest that *Lemna trisulca* may utilize available resources more effectively in specific favorable conditions, although its growth dynamics differ from those of *Lemna minor*.

Lemna minor adjusts its root length based on nutrient availability. Wang and Williams [1990] demonstrated that roots are shorter in environments rich in nitrates and phosphates, suggesting more efficient absorption of available nutrients. The root length of Lemna minor is generally greater compared to Lemna trisulca, indicating that Lemna minor is better adapted for efficient resource utilization in more challenging conditions. Additionally, Radić et al. [2011] showed that water pollution, including increased electrical conductivity, can affect root length by shortening or deforming them, which may limit effective nutrient absorption. Haller et al. [1974] noted that low oxygen concentrations can cause root elongation, promoting better uptake of gases necessary for metabolic processes.

Conversely, strong negative correlations were observed between temperature and the thickness of the duckweed cover as well as sediment coverage. High temperatures may lead to the production of smaller amounts of sediment, potentially indicating environmental stress for this species. Additionally, biomass exhibited a negative correlation with the amount of sediment, suggesting that greater biomass is associated with less sediment coverage. A strong negative correlation between the amount of sediment produced and the oxygen content in the water indicates that greater sediment coverage limits the amount of oxygen, which is consistent with observations regarding the negative impact of high sediment thickness on water quality and the health of aquatic ecosystems [McLay, 1976]. The greater the sediment coverage, the lower the oxygen content, as confirmed by earlier studies regarding the importance of oxygen availability for the growth of aquatic plants [Haller et al., 1974; Wang and Williams, 1990].

However, for *Lemna trisulca*, strong positive correlations were observed between sediment thickness and its coverage, with significantly higher values than for *Lemna minor*. The analysis of sediment production reveals significant differences (F = 56.88, p < 2e-16), which may be influenced by nutrient availability and the number of plants. Greater sediment deposits were found where Lemna developed more intensively [Sree and Appenroth, 2022; Ceschin et al., 2020]. Furthermore, the F values for the number of fronds (67.61, p < 2e-16) and root length (44.15, p < 2e-16) highlight how growth conditions affect the morphological development of Lemna minor. An increased number of fronds and root length facilitate more efficient nutrient uptake, which is crucial for aquatic plants, especially in changing environmental conditions [Haller et al., 1974].

Additionally, the pH value, ranging from 5.12 to 6.88, was found to be suitable for both species, indicating their flexibility and adaptive capabilities to changing habitat conditions [McLay, 1976; Landolt and Kandeler, 1987]. Changes in the number of fronds and the average oxygen content reflect the adaptive responses of both species to resource availability. An increased number of fronds in favourable conditions indicates better photosynthesis and effective resource utilization, as highlighted by studies on the importance of oxygen availability for the growth of aquatic plants [Haller et al., 1974; Wang and Williams, 1990].

Variance analysis emphasizes the significant impact of growth conditions on various growth parameters of *Lemna minor*. The highest coverage values were recorded under long photoperiods and appropriate temperatures, suggesting that the combination of these factors supports intense growth. These results are consistent with the literature indicating that optimal light and temperature conditions are crucial for the growth of duckweed [Appenroth et al., 2010]. Our studies show that *Lemna minor* predominates in terms of coverage and nutrient availability compared to *Lemna trisulca*, especially at higher temperatures and longer photoperiods. According to the findings of Appenroth et al. [2010], *Lemna minor* exhibits greater adaptive capabilities compared to other duckweed species. These capabilities support their resilience and adaptability in the face of unfavourable conditions, indicating greater flexibility in this species to adjust to changing environmental conditions. This could be especially advantageous in bioremediation, where effective uptake of nutrients and other contaminants is crucial [Ziegler et al. 2015].

Moreover, a strong negative correlation between sediment production and oxygen content in water suggests that increased sediment coverage can reduce oxygen availability, negatively impacting water quality [McLay, 1976; Sree and Appenroth, 2022]. *Lemna minor's* ability to reduce sediment formation and enhance water quality through decreased sediment density further highlights its potential for water remediation [Ziegler et al., 2015; Van Dyck et al., 2021]. In comparison, *Lemna trisulca* also plays a role in sediment dynamics, though its response to environmental conditions and its effectiveness in improving oxygen levels may differ. Both species, however, demonstrate valuable traits for bioremediation, with *Lemna minor* potentially offering faster and more efficient results under optimal growth conditions [Ziegler et at. 2023].

CONCLUSIONS

Both *Lemna minor* and *Lemna trisulca* show enhanced growth under higher temperatures and longer photoperiods, with *Lemna minor* achieving superior surface coverage and biomass production under optimal conditions (25 °C and 12-hour photoperiod). This indicates that *Lemna minor* is highly efficient in utilizing favorable environmental conditions, which makes it particularly competitive in nutrient-rich environments.

Both species demonstrated adaptability to variations in water electrical conductivity and pH. With *Lemna minor* achieving higher surface coverage and frond numbers under optimal conditions. However, *Lemna trisulca* showed more stable growth even under more extreme temperature and conductivity conditions, showcasing its ability to maintain growth in harsher environments.

The results suggest that *Lemna minor* has a higher competitive ability under ideal growth conditions, likely due to its more effective utilization of available resources, such as nutrients. This species' rapid biomass production under favorable conditions makes it particularly promising for bioremediation applications, where fast growth and efficient nutrient uptake are critical. Furthermore, *Lemna minor's* ability to adapt to a wide range of physicochemical parameters strengthens its potential for use in diverse aquatic environments.

The study results highlight the adaptive flexibility of both duckweed species, suggesting that each species may have unique applications in the management of aquatic ecosystems. *Lemna minor*, with its high growth rates under stable and favourable conditions, could effectively use in environments where such conditions are maintained, making it ideal for bioremediation tasks that require rapid biomass production. In contrast, *Lemna trisulca* may be more suitable for environments with fluctuating or extreme conditions, where it can maintain stable growth, although with lower biomass and coverage compared to *Lemna minor*.

In conclusion, while *Lemna minor* appears more efficient in resource utilization and biomass accumulation under optimal conditions, *Lemna trisulca* offers advantages in more variable or challenging environments, suggesting complementary roles for both species in bioremediation efforts and ecological management.

However, this study was limited to testing combinations of temperature and photoperiod, which does not fully represent the complexity of real-world bioremediation scenarios. Future research should expand on these findings by investigating additional factors, such as the presence of pollutants, nutrient availability, and interactions with other species. Furthermore, while this study focused on laboratory conditions, the impact of environmental factors like water salinity, pH, and pollution levels on plant growth and contaminant uptake was not explored. These parameters are critical for assessing the true potential of *Lemna minor* and *Lemna trisulca* in practical applications.

REFERENCES

1. Akhtar T. A., Lees H. A., Lampi M. A., Enstone D., Brain R. A., and Greenberg B.M. (2010). Photosynthetic redox imbalance influences flavonoid biosynthesis in Lemna gibba. *Plant, cell and environment*, *33*(7), 1205–1219.
2. Anderson K. E., Lowman Z., Stomp A.M., Chang J. (2011). Duckweed as a feed ingredient in laying hen diets and its effect on egg production and composition. *Int J Poult Sci*, *10*(1), 4–7.
3. Anderson L. and Martin, D. F. (2005). Effect of light quality on the growth of duckweed, Lemna minor L. *Florida Scientist*, 20–24.
4. Appenroth K. J., Krech K., Keresztes A., Fischer W., Koloczek, H. (2010). Effects of nickel on the chloroplasts of the duckweeds Spirodela polyrhiza and Lemna minor and their possible use in biomonitoring and phytoremediation. *Chemosphere*, *78*(3), 216–223.
5. Appenroth K. J., Teller S., Horn M. 1996. Photophysiology of turion formation and germination in Spirodela polyrhiza. *Biologia plantarum*, *38*, 95–106.
6. Ceschin S., Crescenzi M., Iannelli M. A. (2020). Phytoremediation potential of the duckweeds Lemna minuta and Lemna minor to remove nutrients from treated waters. *Environmental Science and Pollution Research*, *27*(13), 15806–15814.
7. Cui W., and Cheng J. J. (2015). Growing duckweed for biofuel production: a review. *Plant biology*, *17*, 16–23.
8. Driever S. M., van Nes E. H., Roijackers R. M. (2005). Growth limitation of Lemna minor due to high plant density. *Aquatic Botany*, *81*(3), 245–251.
9. Fujii Y., Ogasawara Y., Takahashi Y., Sakata M., Noguchi M., Tamura S., Kodama Y. (2020). The cold-induced switch in direction of chloroplast relocation occurs independently of changes in endogenous phototropin levels. *PLoS One*, *15*(5), e0233302.
10. Haller W. T., Sutton D. L., Barlowe W. C. (1974). Effects of salinity on growth of several aquatic macrophytes. *Ecology*, *55*(4), 891–894.
11. Kufel L., Strzałek M., Wysokińska U., Biardzka E., Oknińska S., Ryś K. (2012). Growth rate of duckweeds (Lemnaceae) in relation to the internal and ambient nutrient concentrations–testing the Droop and Monod models. *Pol J Ecol*, *60*(2), 241–249.
12. Landolt E. (1986). Biosystematic investigations in the family of duckweeds (Lemnaceae)(Vol. 2.) The family of Lemnaceae-a monographic study. vol. 1. *Veroff Geobot. Inst. ETH*, *71*, 1-563.
13. Landolt E. and Kandeler R. (1987). The family of Lemnaceae –a monographic study vol. 2. Zürich, Switzerland: Veroffentlichungen des Geobotanischen Institutes der Eidgenossischen Technischen Hochschule, Stiftung Rubel.
14. Lasfar S., Monette F., Millette L., Azzouz A. (2007). Intrinsic growth rate: a new approach to evaluate the effects of temperature, photoperiod and phosphorus–nitrogen concentrations on duckweed growth under controlled eutrophication. *Water research*, *41*(11), 2333–2340.
15. Lemon G. D., Posluszny U., Husband B.C. (2001). Potential and realized rates of vegetative reproduction in Spirodela polyrhiza, Lemna minor, and Wolffia borealis. *Aquatic Botany*, *70*(1), 79–87.
16. Liu M., Yu Z., Jiang L., Hou Q., Xie Z., Ma M., Yu S., Pei H. (2021). Monosodium glutamate wastewater assisted seawater to increase lipid productivity in single-celled algae. *Renewable Energy, 179*, 1793 – 1802, https://doi.org/10.1016/j.renene.2021.08.006
17. Magalhães I.B., Ferreira J., de Siqueira Castro J., Assis L.R.D., Calijuri M.L. (2021). Technologies

for improving microalgae biomass production coupled to effluent treatment: A life cycle approach Algal Research, 57, 102346, https://doi.org/10.1016/j.algal.2021.102346

18. McLay C. L. (1976). The effect of pH on the population growth of three species of duckweed: Spirodela oligorrhiza, Lemna minor and Wolffia arrhiza. *Freshwater Biology*, *6*(2), 125–136.
19. Paolacci S., Jansen M. A., Harrison S. (2018). Competition between Lemna minuta, Lemna minor, and Azolla filiculoides. Growing fast or being steadfast? *Frontiers in Chemistry*, *6*, 207.
20. Pasos-Panqueva J., Baker A., Camargo-Valero M. A. (2024). Unravelling the impact of light, temperature and nutrient dynamics on duckweed growth: A meta-analysis study. *Journal of Environmental Management*, *366*, 121721.
21. Puiseux-Dao S. (2018). *Phytoplankton model in ecotoxicology Aquatic Ecotoxicology Fundamental Concepts and Methodologies*, 2, 163–186, https://doi.org/10.1201/9781351069854
22. Radić S., Stipaničev D., Cvjetko P., Rajčić M. M., Širac S., Pevalek-Kozlina B., Pavlica M. (2011). Duckweed Lemna minor as a tool for testing toxicity and genotoxicity of surface waters. *Ecotoxicology and environmental safety*, *74*(2), 182–187.
23. Sender J. and Różańska-Boczula M. (2024). Preliminary studies of selected Lemna species on the oxygen production potential in relation to some ecological factors. *PeerJ*, *12*, e17322.
24. Sree K. S., and Appenroth K. J. (2022). Starch accumulation in duckweeds (Lemnaceae) induced by nutrient deficiency. *Emirates Journal of Food and Agriculture*, *34*(3), 204–212.
25. Sree K. S., Bog M., Appenroth K. J. (2016). Taxonomy of duckweeds (Lemnaceae), potential new crop plants. *Emirates Journal of Food and Agriculture (EJFA)*, *28*(5).
26. Strzałek M., and Kufel L. (2021). Light intensity drives different growth strategies in two duckweed species: Lemna minor L. and Spirodela polyrhiza (L.) Schleiden. *PeerJ*, *9*, e12698.
27. Sun Z., Guo W., Yang J., Zhao X., Chen Y., Yao L., Hou H. (2020). Enhanced biomass production and pollutant removal by duckweed in mixotrophic conditions. *Bioresource Technology*, *317*, 124029.
28. Tippery N. P., and Les D. H. (2020). Tiny plants with enormous potential: phylogeny and evolution of duckweeds. *The duckweed genomes*, 19–38.
29. Toyama T., Hanaoka T., Tanaka Y., Morikawa M., Mori K. (2018). Comprehensive evaluation of nitrogen removal rate and biomass, ethanol, and methane production yields by combination of four major duckweeds and three types of wastewater effluent. Bioresource technology, *250*, 464–473.
30. Van Dyck I., Vanhoudt N., i Batlle J. V., Horemans N., Nauts R., Van Gompel A., ... and Vangronsveld J. (2021). Effects of environmental parameters on Lemna minor growth: An integrated experimental and modelling approach. *Journal of environmental management*, *300*, 113705.
31. Velichkova K., Sirakov I., Staykov Y. (2018). Integrated use of two microalgal species for the treatment of aquaculture effluent and biomass production. *Environmental Engineering and Management Journal, 17*(7), 1575–1581, https://doi.org/10.30638/eemj.2018.156
32. Walsh É., Kuehnhold H., O'Brien S., Coughlan N. E., Jansen M. A. (2021). Light intensity alters the phytoremediation potential of Lemna minor. *Environmental Science and Pollution Research*, *28*, 16394–16407.
33. Wang W., and Williams J. M. (1990). The use of phytotoxicity tests (common duckweed, cabbage, and millet) for determining effluent toxicity. *Environmental monitoring and assessment*, *14*, 45–58.
34. Xu J, Cheng J.J, Stomp A.M. (2012). Growing Spirodela polyrrhiza in swine wastewater for the production of animal feed and fuel ethanol: a pilot study. *Clean Soil Air Water 40*(7), 760–765
35. Xu J, Cui W, Cheng J.J, Stomp A.M. (2011). Production of high-starch duckweed and its conversion to bioethanol. Biosystems Eng. 110(2), 67–72.
36. Xu J., Cui W., Cheng, J. 2012. Temperature and Light Effects on Growth of Lemna minor. Environmental and Experimental Botany, 78, 39–45.
37. Xu Y., Ma S., Huang M., Peng M., Bog M., Sree K. S., ... and Zhang J. (2015). Species distribution, genetic diversity and barcoding in the duckweed family (Lemnaceae). *Hydrobiologia*, *743*, 75–87.
38. Ziegler P., Adelmann K., Zimmer S., Schmidt C., Appenroth K. J. (2015). Relative in vitro growth rates of duckweeds (L emnaceae)–the most rapidly growing higher plants. *Plant biology, 17*, 33–41.
39. Ziegler P., Appenroth K. J., Sree K. S. (2023). Survival strategies of duckweeds, the world's smallest Angiosperms. Plants, *12*(11), 2215.