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Integrated effects of light intensity, spectral composition and photoperiod fragmentation on duckweed growth and nutrient removal

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Abstract

Duckweeds are widely used in wastewater treatment and biomass production systems; however, the combined effects of light intensity, spectral composition and photoperiod structure on their performance remain insufficiently understood. This study evaluated how these light parameters influence growth and nutrient removal in three duckweed species (*Lemna minor*, *Landoltia punctata* and *Spirodela polyrhiza*) cultivated under controlled conditions. Light intensity (50–1220 $\mu\text{mol m}^{-2} \text{s}^{-1}$), spectral composition (blue, green, red, white and mixed spectra), and fragmented photoperiod regimes (12L:12D to 1L:1D) were examined. Spectral and photoperiod treatments were conducted at a constant daily light integral ($\sim 12.96 \text{ mol m}^{-2} \text{d}^{-1}$), enabling isolation of light-quality and temporal effects from total photon dose. Relative growth rate (RGR), dry biomass and nitrogen (N- NO_3) and phosphorus (P- PO_4) removal were assessed. Growth responses showed saturation with increasing light intensity, with RGR saturating at lower levels (160–296 $\mu\text{mol m}^{-2} \text{s}^{-1}$) than dry biomass (~ 343 –440 $\mu\text{mol m}^{-2} \text{s}^{-1}$). Red-enriched white light generally enhanced growth and nutrient removal across species, whereas fragmented photoperiods reduced growth despite identical daily light integral.

Keywords

duckweed, light intensity, light quality, intermittent photoperiod, plant growth, nutrient removal

19 INTRODUCTION

20 Nature-based solutions, including constructed wetlands, have gained increased attention as
21 sustainable and energy-efficient solutions for wastewater treatment and nutrient recovery [1].

22 In such systems, macrophytes play a key role in stabilizing treatment performance, assimilating
23 nutrients, and influencing associated microbial communities [2–4]. Among macrophytes,
24 floating plants, especially species from the duckweed family (*Lemnaceae*), are of particular
25 interest due to their rapid growth, simple morphology, and high tolerance to variable
26 environmental conditions [5].

27 Duckweeds are among the smallest vascular plants, consisting of 37 species belonging to five
28 genera: *Lemna*, *Spirodela*, *Landoltia*, *Wolffia*, and *Wolffiella* [6]. They are widely distributed
29 worldwide, commonly colonizing inland surface waters, including ponds, lakes, canals, and
30 reservoirs [7, 8]. Duckweeds reproduce primarily through clonal propagation and can exhibit
31 exceptionally high growth rates under optimal conditions, doubling their biomass within 2–3
32 days and forming dense surface mats [9, 10].

33 Although commonly associated with constructed wetland systems, duckweeds are highly
34 versatile, with applications including animal feed and food additives [11]. Furthermore,
35 biomass produced in constructed wetland systems can be valorized as a co-substrate in
36 fermentation processes, supporting circular economy concepts [12, 13]. Duckweed-based
37 systems are also associated with low energy demand and relatively cost-efficient operation,
38 which enhances their applicability in sustainable water treatment technologies [5].

39 Despite their numerous advantages, the performance of duckweed-based systems is sensitive
40 to environmental conditions. Plant growth and nutrient uptake are species-specific and depend
41 on abiotic factors, including the source and type of biogenic compounds, temperature, pH and
42 growth surface availability [14, 15]. In this context, light also plays a particularly important
43 role, acting as the primary energy source for plants, driving photosynthesis and thus enabling

44 inorganic carbon assimilation and the synthesis of organic compounds, while also providing
45 key regulatory signals [16]. The growth and development of plants depend on controlling light
46 parameters, including light quantity, defined by intensity and photoperiod duration, as well as
47 light quality, determined by the spectral composition of the applied radiation.

48 Despite the growing interest in duckweed-based cultivation and wastewater treatment systems,
49 the combined effects of light intensity, spectral composition, and photoperiod structure on
50 duckweed growth and nutrient removal remain insufficiently understood. Previous studies have
51 typically focused on single light factors, such as intensity or spectrum, while the temporal
52 distribution of light and its interaction with total light dose have received comparatively little
53 attention, particularly in multi-species systems. Moreover, the lack of studies conducted under
54 controlled and comparable daily light integral (DLI) conditions limits the interpretation of light-
55 response mechanisms in duckweed.

56 Therefore, the present study aimed to provide a comprehensive evaluation of how light
57 intensity, spectral composition, and fragmented photoperiod regimes jointly influence the
58 growth performance and nutrient removal efficiency of three duckweed species (*Lemna minor*,
59 *Landoltia punctata*, and *Spirodela polyrhiza*) under controlled conditions. By maintaining a
60 constant DLI in the spectral and photoperiod experiments, this study specifically isolates the
61 effects of light quality and temporal light distribution from total photon input, enabling a more
62 mechanistic interpretation of duckweed responses to lighting regimes.

63 In this context, we hypothesized that light regime characteristics, including intensity, spectral
64 composition, and photoperiod structure, would differentially influence duckweed growth and
65 nutrient uptake, and that maintaining a constant daily light integral would allow the separation
66 of total light dose effects from temporal light distribution effects.

67

68

MATERIALS AND METHODS

Plant material and pre-culture

Lemna minor, *Landoltia punctata*, and *Spirodela polyrhiza* plants were purchased from aquatic plant supplier (Krewetki Ketrab, Poland; Tropica, Denmark). Cultivation was carried out using Steinberg's medium [17] in polypropylene cup containers and Petri dishes. The plants were kept in climate chamber at 25 °C, provided by Osram Fluora L36W fluorescent lamps (Osram, Germany), with light intensity of 40–50 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and a photoperiod of 12 h light:12 h dark.

Experimental conditions

In each experiment, plants were grown in polypropylene cup containers loosely covered with transparent polyethylene terephthalate lids, each containing 50 ml of Steinberg's medium. Each treatment consisted of four replicates. A defined number of fronds were introduced into each cup: 10 fronds of *L. minor* (5 plants per cup), 10 fronds of *L. punctata* (4 plants per cup), and 6 fronds of *S. polyrhiza* (3 plants per cup). The experiments were conducted using the Chihiros WRGB II Pro Series LED Lighting System (Chihiros Aquatic Studio, China) which allows independent adjustment of spectral composition and photon flux. Light intensity was quantified as photosynthetic photon flux density (PPFD, $\mu\text{mol m}^{-2} \text{s}^{-1}$) within the photosynthetically active radiation (PAR, 400–700 nm) range. PPFD levels were measured and calibrated using StellarNet BLACK-Comet spectrometer and the SpectraWiz Software (StellarNet, United States). The photoperiod was maintained as 12 h of light and 12 h of darkness per day, at temperature of 23–26°C.

For the spectral composition and photoperiod structure experiments, the daily light integral (DLI) was maintained at approximately 12.96 $\text{mol m}^{-2} \text{d}^{-1}$ ($300 \mu\text{mol m}^{-2} \text{s}^{-1} \times 12 \text{ h}$) to ensure that observed differences in growth and nutrient removal resulted from light quality and temporal light distribution rather than total photon dose. In the light intensity experiment, DLI

varied proportionally with PPFD, as light intensity was the primary experimental variable [18].

Plants were incubated for 14 days, except for the light intensity experiment, which lasted 7 days. To ensure uniform illumination, the positions of the plants under the lamps were randomized daily. Images were taken at regular time intervals to monitor plant growth throughout the experiments. Photographs were obtained using a OnePlus 12 smartphone under standardized lighting, and frond number and surface area were quantified using ImageJ software (National Institutes of Health, United States). At the end of the experiment the dry weight (105°C) was determined.

According to OECD 221 standard [17], the relative growth rates (RGR) of frond number and frond surface area were calculated after 7 days, based on Equation 1.

$$RGR = \frac{\ln(N_t) - \ln(N_0)}{t} [\text{day}^{-1}] \quad (1)$$

where N_t – number of fronds or fronds surface area at time t (at the specific time point), N_0 – number of fronds or fronds surface area at the start of the experiment, t – time period from 0 to t .

Effect of light intensity

The light intensity experiment was conducted under white light (415–685 nm, with emission peaks at 450, 520, and 630 nm, Supplementary Fig. S1) at six PPFD levels: 50, 210, 390, 610, 960, and 1220 $\mu\text{mol m}^{-2} \text{s}^{-1}$. The light saturation point (I_{sat}), defined as the light intensity corresponding to 90% of the fitted maximum RGR (RGR_{max}) or fitted maximum dry biomass (DW_{max}), was estimated by nonlinear regression analysis using a Gompertz model (Eq. 2), fitted to the RGR–light response curve with the Levenberg-Marquardt algorithm implemented in OriginPro 2025b (OriginLab Corporation, United States).

$$Y(I) = Y_{\text{max}} \exp[-\exp(-k(I - x_c))] [\text{day}^{-1}] \text{ or } [\text{mg}] \quad (2)$$

where: I – light intensity [$\mu\text{mol m}^{-2} \text{s}^{-1}$], $Y(I)$ – the response at light intensity I (RGR, day^{-1} , or

dry biomass, mg), Y_{max} – RGR_{max} or DW_{max} (plateau), k – slope parameter controlling the rate at which the response approaches the plateau, x_c – location parameter describing the horizontal shift of the curve.

Effect of light spectra

Six different spectral treatments were applied at a constant PPFD of 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$, selected as near-saturating light level based on the results of the light intensity experiment. Three monochromatic light spectra were applied: blue light (BL; 410–500 nm, peak emission at 450 nm), green light (GL; 465–600 nm, peak emission at 520 nm), red light (RL; 580–670 nm, peak emission at 630 nm). In addition to monochromatic light treatments, polychromatic spectra were tested, namely white light (WL; 415–685 nm, with emission peaks at 450, 540, and 630 nm) and selected combined spectra were tested, including 70/30% WL/BL (WBL) and WL/RL (WRL) splits (Supplementary Fig. S2).

Effect of photoperiod structure

The photoperiod experiment was conducted under WL at a constant PPFD of 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Five photoperiod variants with different light–dark splitting patterns were applied, each consisting of alternating light (L) and dark (D) phases, with a total of 12 h of light and 12 h of darkness per day: 12L:12D (12 h light:12 h dark), 6L:6D, 3L:3D, 2L:2D, and 1L:1D.

Nitrogen and phosphorus removal

After 14 days of incubation (at the end of experiment) water samples were collected and in each, the volume was then adjusted to the initial volume, followed by filtration through 0.45 μm Nylon filters (Chemland, Poland). N-NO₃ and P-PO₄ concentration were analyzed using Ion Chromatography Dionex Aquion (Thermo Scientific, Poland). Initial concentrations (C_0) were 88.1 mg N-NO₃ L⁻¹ (390 mg L⁻¹ NO₃⁻) and 30.3 mg P-PO₄ L⁻¹ (93 mg L⁻¹ PO₄³⁻). The removal efficiencies of N-NO₃ and P-PO₄ were calculated according to Eq. 3.

$$\text{RE} = \frac{C_0 - C_t}{C_0} \cdot 100\% [\%] \quad (3)$$

where RE – removal efficiencies of either N-NO₃ or P-PO₄ [%], C₀ – N-NO₃ and P-PO₄ concentrations at the start of the experiment [mg L⁻¹], C_t – N-NO₃ and P-PO₄ concentrations at the time t [mg L⁻¹].

Statistical analysis

Each experimental variant included four replicates. The results are presented as mean ± standard deviation. Statistical analysis was performed using Statistica 14 software (StatSoft Polska, Poland) and OriginPro 2025b (OriginLab Corporation, United States). The assumptions of normality (Shapiro–Wilk test) and homogeneity of variances (Levene test) were verified prior to analysis. One-way analysis of variance (ANOVA) followed by Tukey's Honest Significant Difference (HSD) post hoc test was applied at a significance level of $\alpha = 0.05$. When the assumption of homogeneity of variances was violated, Welch's ANOVA followed by Games–Howell post hoc comparisons was used ($\alpha = 0.05$). In the graphs, bars marked with the same letters do not differ significantly, whereas different letters indicate statistically significant differences.

RESULTS AND DISCUSSION

Effect of light intensity

Fig. 1 presents the RGR values calculated based on the number of fronds and frond surface area, together with the corresponding dry biomass values, across the tested light intensities. Consistent with previous reports, *L. minor* exhibited the highest growth rates, whereas *L. punctata* and *S. polyrhiza* showed lower and relatively similar RGR values. Mean RGR values ($\pm$ SD) based on frond number ranged from 0.265 ± 0.018 to 0.465 ± 0.021 for *L. minor*, 0.180 ± 0.019 to 0.371 ± 0.013 for *L. punctata*, and 0.192 ± 0.022 to 0.356 ± 0.018 for *S. polyrhiza*. A comparable pattern was observed when RGR was calculated using frond surface area, with mean values ($\pm$ SD) ranging from 0.317 ± 0.014 to 0.466 ± 0.012 in *L. minor*, 0.200 ± 0.019 to

166 0.384 $\pm$ 0.013 in *L. punctata*, and 0.164 $\pm$ 0.016 to 0.345 $\pm$ 0.022 in *S. polyrhiza*. Overall, the
167 observed growth rates remained within the ranges previously reported for these species [19].
168 Dry biomass showed a similar increasing trend with light intensity, with the greatest values
169 observed at the highest tested intensities. Mean dry mass ranged from 3 to 14 mg for *L. minor*,
170 3 to 13 mg for *L. punctata*, and 5 to 23 mg for *S. polyrhiza*.
171 All three species showed a comparable increase in RGR between 50 and 390 $\mu\text{mol m}^{-2} \text{s}^{-1}$,
172 whereas at higher intensities their responses varied. While *L. minor* continued to increase
173 slightly up to the maximum intensity tested, *L. punctata* approached a plateau beyond 390 μmol
174 $\text{m}^{-2} \text{s}^{-1}$. In contrast, *S. polyrhiza* reached peak RGR at 610 $\mu\text{mol m}^{-2} \text{s}^{-1}$, followed by slightly
175 lower values at higher intensities. Previous studies demonstrated similar species-specific
176 responses to increasing light intensity, showing that *S. polyrhiza* growth declined at the highest
177 intensities, while *L. minor* continued to exhibit comparatively higher growth rates [20, 21].

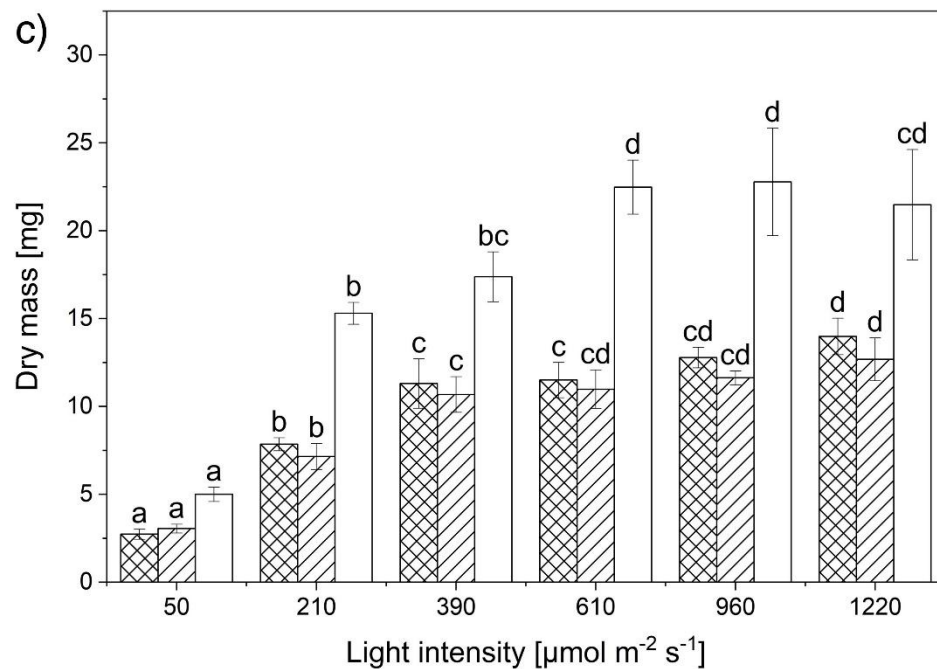
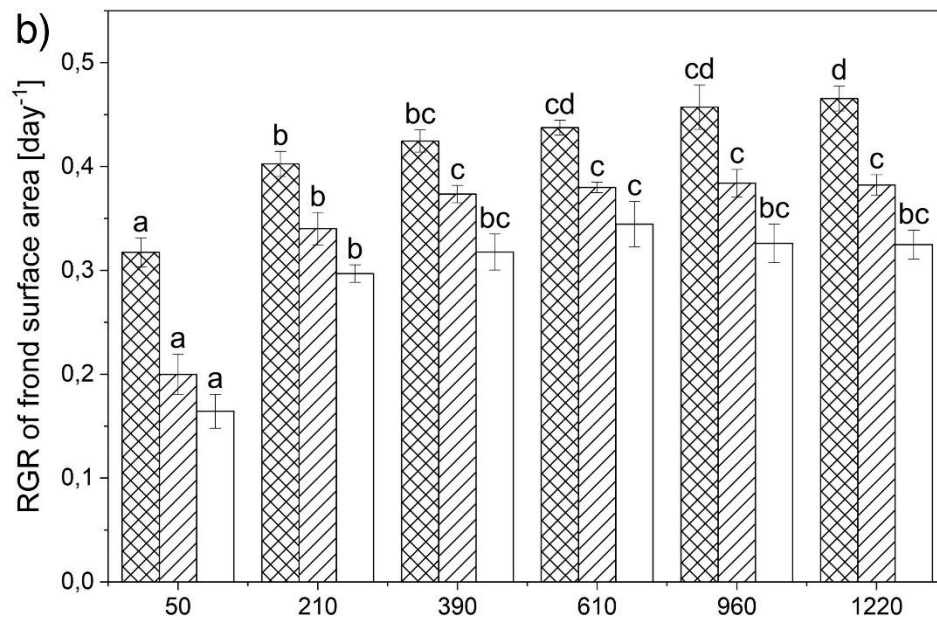
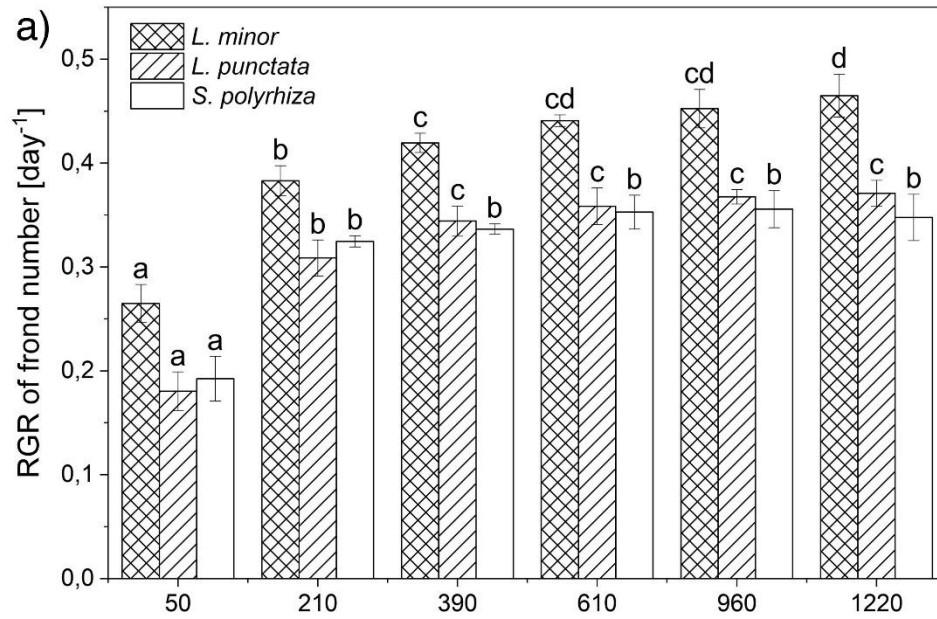


Figure 1. RGR values calculated based on (a) frond number and (b) frond surface area, together with (c) dry biomass of *L. minor*, *L. punctata*, and *S. polyrhiza* under increasing light intensity (50–1220 $\mu\text{mol m}^{-2} \text{s}^{-1}$). Values represent means $\pm$ SD ($n = 4$). Different letters indicate significant differences among light intensities within each species (Tukey HSD, $p < 0.05$).

I_{sat} values corresponding to 90% of RGR_{max} or DW_{max} are presented in Table 1 and the corresponding Gompertz fit parameters are provided in Supplementary Table S1. The 90% threshold is frequently used in studies of photosynthetic light-response curves [22–24] and was applied here as a practical approximation of the I_{sat} for plant growth rates. Although the obtained values are broadly consistent with those reported in the literature, direct comparison requires consideration of several factors. Regarding light conditions, light quantity depends on both light intensity and photoperiod duration and is frequently expressed as the daily light integral (DLI), which integrates these factors [25]. Consequently, variations in photoperiod may influence I_{sat} values. Longer photoperiods may reduce the light intensity required to achieve light saturation, whereas shorter photoperiods may necessitate higher light intensities to reach a comparable daily light dose. Nevertheless, this effect may vary, as plant growth rates also depend on other environmental conditions, including nutrient composition and temperature. The highest growth rates are typically observed under well-optimized mineral media [26]. Moreover, I_{sat} increases with temperature [27], with maximal growth observed at approximately 20–30 °C [14].

Accordingly, reported I_{sat} values for *L. minor* range from 250–300 $\mu\text{mol m}^{-2} \text{s}^{-1}$ under a 12:12 (L/D) photoperiod [28] up to 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$ under a 16:8 (L/D) regime [29]. Comparable values have been also reported for other *Lemna* species, including 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for *L. minuta* (16:8 L/D) [29] and 200–400 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (12:12 L/D) or 110 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (continuous light) for *L. aequinoctialis* [30]. For *S. polyrhiza*, photosynthetic light saturation at roughly 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$ has been documented [31], whereas *L. punctata* has been described to exhibit a

broader saturation range of 600–1200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ [32]. However, growth and photosynthesis may reach saturation at different light intensities, as the light level required for growth saturation is typically lower than that required for photosynthesis [27]. Nonetheless, *S. polyrhiza* has been shown to reach growth saturation at approximately half the light intensity necessary for *L. minor* (ca. 150 vs. 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$) [33]. To the best of the authors' knowledge, growth-based I_{sat} values have not yet been reported for *L. punctata*. However, in recent meta-analysis by Pasos-Panqueva et al. (2024) [14], the authors suggest that, regardless of species and photoperiod, duckweed growth rates generally increase with light intensity up to approximately 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$, while further increases up to 800 $\mu\text{mol m}^{-2} \text{s}^{-1}$ do not substantially increase RGR. Similarly, the analysis indicates that optimal growth occurs within a range of approximately 5–20 $\text{mol m}^{-2} \text{d}^{-1}$ when expressed as DLI.

Table 1. I_{sat} values (expressed in $\mu\text{mol m}^{-2} \text{s}^{-1}$) corresponding to 90% of RGR_{max} for frond number and frond surface area, and to 90% of DW_{max} for *L. minor*, *L. punctata*, and *S. polyrhiza*.

I_{sat} ($\mu\text{mol m}^{-2} \text{s}^{-1}$) for:	Species		
	<i>L. minor</i>	<i>L. punctata</i>	<i>S. polyrhiza</i>
RGR – frond number	296 $\pm$ 35	286 $\pm$ 21	160 $\pm$ 17
RGR – frond surface area	274 $\pm$ 67	220 $\pm$ 7	205 $\pm$ 17
Dry biomass	431 $\pm$ 35	440 $\pm$ 44	343 $\pm$ 49

I_{sat} for dry biomass was reached at higher light intensities than for RGR (Table 1), indicating that biomass accumulation continued to increase even after RGRs plateaued. A similar pattern was described by Landolt and Kandeler (1987) [27], who reported that dry weight continued to increase at light intensities exceeding the saturation point for growth.

Similar light-dependent responses have been documented in recent studies and are attributed to adjustments in plant biochemical profiles. Under higher light intensities, plants exhibit lower levels of photosynthetic pigments, increased concentrations of photoprotective carotenoids (e.g., zeaxanthin), altered protein content, and enhanced starch accumulation [34–36]. For instance, Yin et al. (2015) [30] reported higher starch content in *L. aequinoctialis* under elevated light intensity, particularly during the initial days of incubation, regardless of photoperiod. Increased starch accumulation could contribute to the greater dry biomass observed in our experiment at higher light levels.

Based on the results described above, $300 \mu\text{mol m}^{-2} \text{s}^{-1}$ was selected for subsequent experiments, as this light intensity closely matched the mean RGR-based I_{sat} values across all three species while simultaneously simplified subsequent experimental design through the use of a common saturating light level. At intensities above this threshold, further increases in both RGR and dry biomass were minor and mostly statistically non-substantial.

Effect of light spectra

The effects of light spectra on RGR and dry biomass in the tested duckweed species are shown in Fig. 2. Although the plants differed in their growth responses, similar trends were observed across all three species.

For *L. minor*, frond number-based RGR increased from 0.346 ± 0.015 – $0.346 \pm 0.017 \text{ day}^{-1}$ under WBL and WL to $0.390 \pm 0.015 \text{ day}^{-1}$ under WRL, while surface area-based RGR increased from 0.342 ± 0.009 – $0.361 \pm 0.018 \text{ day}^{-1}$ to $0.401 \pm 0.015 \text{ day}^{-1}$. A similar pattern was observed in *L. punctata* for surface area-based RGR (0.293 ± 0.004 – 0.302 ± 0.006 to $0.327 \pm 0.012 \text{ day}^{-1}$), whereas frond number-based RGR showed no significant differences among treatments (0.315 ± 0.011 , $0.324 \pm 0.012 \text{ day}^{-1}$, $0.330 \pm 0.010 \text{ day}^{-1}$ for WBL, WL and WRL, $p > 0.05$). In *S. polyrhiza*, both parameters followed a comparable trend, with frond

number-based RGR increasing from 0.315 ± 0.012 – 0.316 ± 0.007 to 0.350 ± 0.020 day⁻¹ and surface area-based RGR from 0.274 ± 0.010 – 0.279 ± 0.012 to 0.312 ± 0.014 day⁻¹. Despite the differences observed in RGR, dry biomass did not differ significantly ($p > 0.05$) among the tested polychromatic spectra within species, ranging from 36 – 39 mg in *L. minor*, 35 – 42 mg in *L. punctata*, and 49 – 58 mg in *S. polyrhiza*.

Species-specific responses were also observed under monochromatic spectra. In *S. polyrhiza*, RGR increased from BL to GL and RL for parameters (0.309 ± 0.012 to 0.339 ± 0.012 day⁻¹ for frond number; 0.260 ± 0.011 to 0.302 ± 0.015 day⁻¹ for surface area). In *L. minor*, differences were smaller for frond number (0.321 ± 0.017 – 0.353 ± 0.017 day⁻¹), whereas surface area-based RGR increased from 0.321 ± 0.008 under BL to 0.375 ± 0.009 – 0.371 ± 0.029 day⁻¹ under GL and RL. In *L. punctata* there were no significant ($p > 0.05$) differences and RGR ranged from 0.280 ± 0.010 – 0.298 ± 0.008 day⁻¹ (frond number) and 0.266 ± 0.003 – 0.291 ± 0.012 day⁻¹ (surface area). Dry biomass under monochromatic spectra showed limited variation compared with RGR, ranging from 29 – 35 mg in *L. minor*, 29 – 34 mg in *L. punctata*, and 46 – 49 mg in *S. polyrhiza*.

Compared with monochromatic spectra, WRL resulted in higher RGR values across species, whereas WL and WBL produced species-dependent responses. In contrast, dry biomass remained relatively similar across both monochromatic and polychromatic spectra.

Based on the concept of photosynthetically active radiation, photons with wavelengths between 400 and 700 nm are assumed to have the same photosynthetic potential. However, spectral composition also provides wavelength-specific signals perceived not only by chlorophylls but also by other pigments involved in light harvesting and photoprotection (e.g., carotenoids, anthocyanins), as well as by specialized photoreceptors (e.g., phytochromes, cryptochromes, phototropins), ultimately modulating plant growth and morphology. In duckweed, light quality has been linked to the regulation of developmental responses such as flowering and turion

273 induction and germination in *S. polyrhiza*. Overall, spectral composition may consequently
274 shape duckweed growth and biomass yield and influence its nutrient-uptake dynamics.
275 Petersen et al. (2022) [37] reported no differences in the growth response of *L. minor* and *W.*
276 *hyalina* under mixed white-light spectra with varying red:blue ratios, but no monochromatic
277 treatments were included. Gallego et al. (2021) [38] showed comparable growth of *L. punctata*
278 in fluorescent white, LED white, and blue light, whereas Li et al. (2022) [39] observed higher
279 growth of this species under red light compared with blue and white. For *S. polyrhiza*, Xu et al.
280 (2019) [40] reported greater biomass accumulation under red light but higher starch
281 accumulation under blue light, and both Xu et al. (2019) and Li et al. (2022) [39] suggested that
282 optimized red-to-blue ratios may simultaneously support growth and starch accumulation. In
283 contrast, Zhong et al. (2022) [41] reported only minor growth differences between red and blue
284 light in *S. polyrhiza* (with slightly reduced growth under white light), but clear physiological
285 differences, including higher carotenoid content and amino acid levels under blue light, higher
286 starch accumulation and chloroplast deformation under red light, and altered gene expression
287 between spectra.

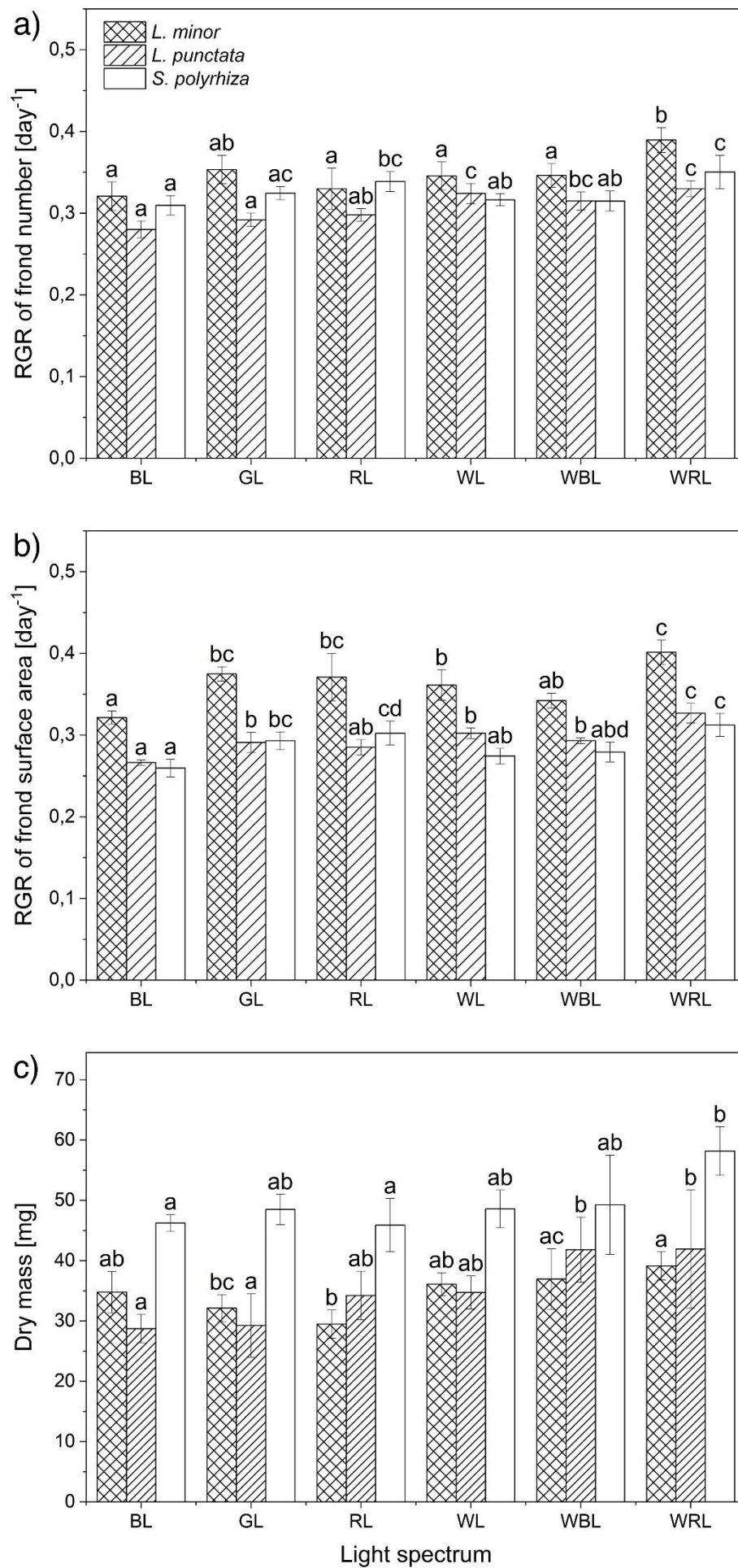
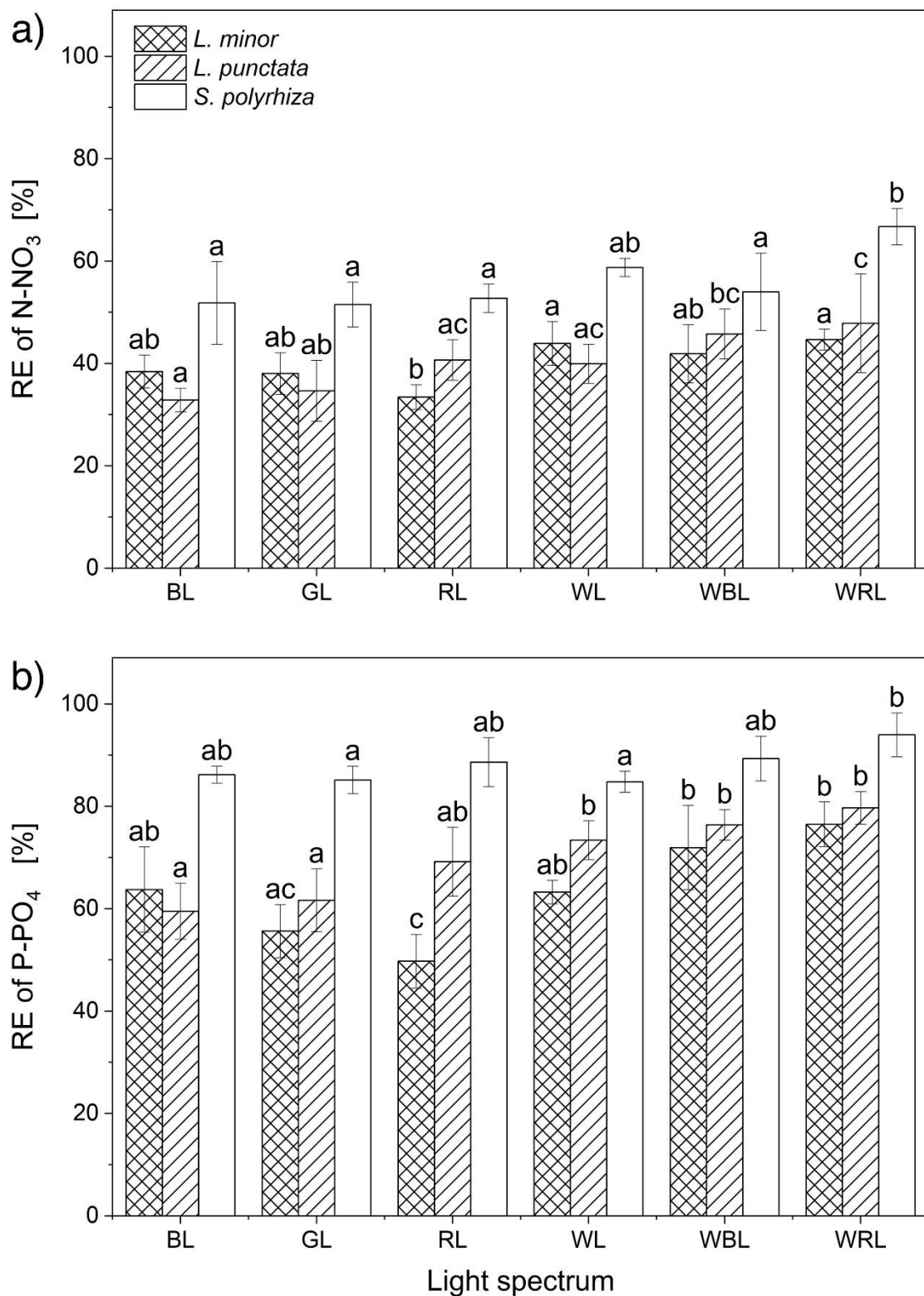


Figure 2. RGR values calculated based on (a) frond number and (b) frond surface area, together with (c) dry biomass of *L. minor*, *L. punctata*, and *S. polyrhiza* under different light spectra (BL, GL, RL, WL, WBL and WRL). Values represent means $\pm$ SD ($n = 4$). Different letters indicate significant differences among light spectra within each species (Tukey HSD, $p < 0.05$).

For nitrogen, differences among spectra were generally moderate (Fig. 3a). In *L. minor*, removal ranged approximately 33 – 45%, with significantly lower values under RL ($33.41 \pm 2.41\%$) compared with WRL ($44.65 \pm 2.03\%$) ($p = 0.0067$). In *S. polyrhiza*, the influence of light spectrum was most evident. The WRL treatment achieved the highest removal ($66.72 \pm 3.56\%$) and was greater than the lowest-performing spectra (ranging 52 – 54%). In *L. punctata*, WRL achieved the highest removal ($47.83 \pm 9.65\%$) and was significantly higher than BL ($32.83 \pm 2.30\%$, $p = 0.0146$) and GL ($34.63 \pm 5.97\%$, $p = 0.0368$), whereas RL, WL, and WBL were mid-range (40–46%). Overall, the highest nitrogen removal frequently occurred under WRL, most clearly in *S. polyrhiza*.

For phosphorus (Fig. 3b), removal patterns were broadly consistent with those observed for nitrogen. In *L. minor*, removal ranged from $49.72 \pm 5.26\%$ under RL to $76.49 \pm 4.37\%$ under WRL, with RL significantly lower than WRL ($p = 0.0002$) and WBL ($p = 0.0008$), while the remaining spectra showed comparable performance. In *L. punctata*, phosphorus removal was significantly lower under BL ($59.48 \pm 5.48\%$) and GL ($61.63 \pm 6.16\%$) than under the polychromatic spectra ($p < 0.05$), with WRL showing the highest removal ($79.70 \pm 3.18\%$). In *S. polyrhiza*, phosphorus removal was consistently high (85–94%), with WRL showing the highest removal ($93.98 \pm 4.27\%$) and being significantly higher than BL ($86.17 \pm 1.68\%$, $p = 0.0243$) and WL ($84.79 \pm 2.06\%$, $p = 0.0180$).



312

313 **Figure 3.** RE of (a) N-NO₃ (b) P-PO₄ of *L. minor*, *L. punctata*, and *S. polyrhiza* under different
 314 light spectra (BL, GL, RL, WL, WBL and WRL). Values represent means ± SD (n = 4).
 315 Different letters indicate significant differences among light spectra within each species (Tukey
 316 HSD; Games–Howell for N-NO₃ RE of *S. polyrhiza*, p < 0.05).

Literature on nutrient uptake under different light spectra in duckweed-based systems remains scarce, but the available studies suggest that light quality can influence nitrogen and phosphorus removal. Xu et al. (2019) [40] showed that in *S. polyrhiza* white, red and red–blue spectra promoted faster nitrogen and phosphorus uptake than blue light. Similarly, Li et al. (2022) [39] reported that white light and a red-to-blue ratio of 2:1 supported the fastest uptake of ammonium nitrogen (N–NH₄) and phosphorus, compared with blue light and other red-to-blue ratios. Gallego et al. (2021) [38] also found that white light showed the most effective overall nitrogen and phosphorus removal, whereas blue performed worst for nitrogen and fluorescent white for phosphorus. Overall, these studies suggest a higher removal potential under white and red light, although in our experiments plant responses were not consistent across treatments, indicating that the effects may be system-specific.

Effect of photoperiod structure

The changes in plant growth under different intermittent photoperiod regimes are presented in Fig. 4. Overall, similar trends were observed across all three species, with the highest RGR values recorded under the standard 12L:12D regime and the lowest under 1L:1D, although differences among intermittent photoperiod variants were not statistically significant ($p > 0.05$). For *L. minor*, RGR based on frond number ranged from 0.397 ± 0.015 to 0.423 ± 0.008 day⁻¹, while values based on frond surface area varied between 0.398 ± 0.007 and 0.442 ± 0.016 day⁻¹. In *L. punctata*, frond number-based RGR ranged from 0.292 ± 0.015 to 0.335 ± 0.013 day⁻¹, while surface area-based RGR varied between 0.289 ± 0.014 and 0.350 ± 0.015 day⁻¹. For *S. polyrhiza*, the corresponding ranges were 0.305 ± 0.015 to 0.354 ± 0.011 day⁻¹ for frond number and 0.270 ± 0.011 to 0.315 ± 0.012 day⁻¹ for surface area. In addition to RGR, dry biomass (Fig. 4c) and nutrient removal (Fig. 5) showed consistent (although mostly non-significant) trends across photoperiod variants. Dry mass ranged 35 – 39

342 mg in *L. minor*, 28 – 33 mg in *L. punctata*, and 45 – 52 mg in *S. polyrhiza*. While differences
343 in dry mass were not statistically significant ($p > 0.05$), it showed a gradual increase under less
344 fragmented photoperiod regimes, consistent with the higher RGR values observed under the
345 standard 12L:12D cycle. Importantly, dry mass was determined one week after RGR, so the
346 two parameters may not align directly. Consequently, treatments promoting faster early growth
347 could also drive earlier nutrient uptake, which is reflected in the tendency for higher N-NO₃
348 and P-PO₄ removal under less fragmented regimes (6L:6D, 12L:12D) (Fig. 5). Since all
349 photoperiod treatments were adjusted to provide the same daily light integral ($\sim 12.96 \text{ mol m}^{-2}$
350 d^{-1}), the observed differences in growth and nutrient removal can be attributed to photoperiod
351 fragmentation rather than differences in total light availability.

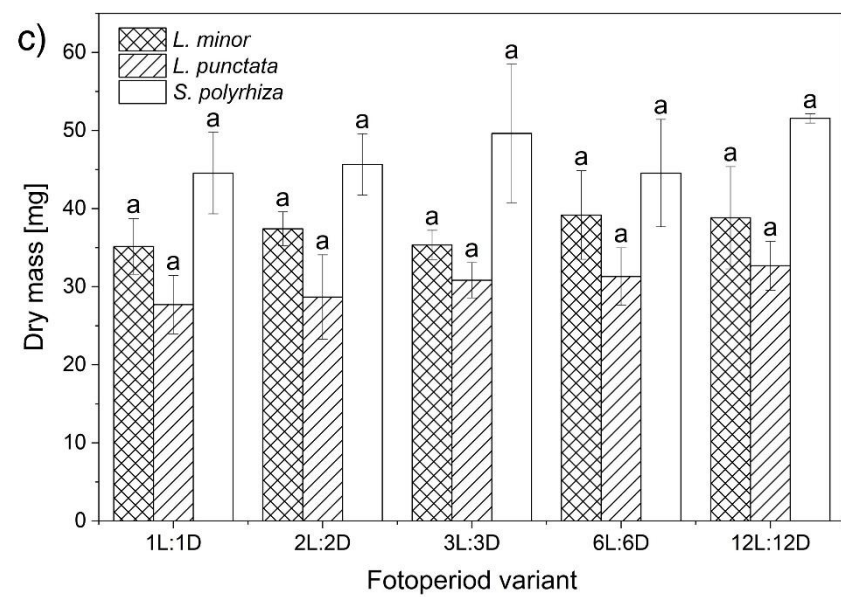
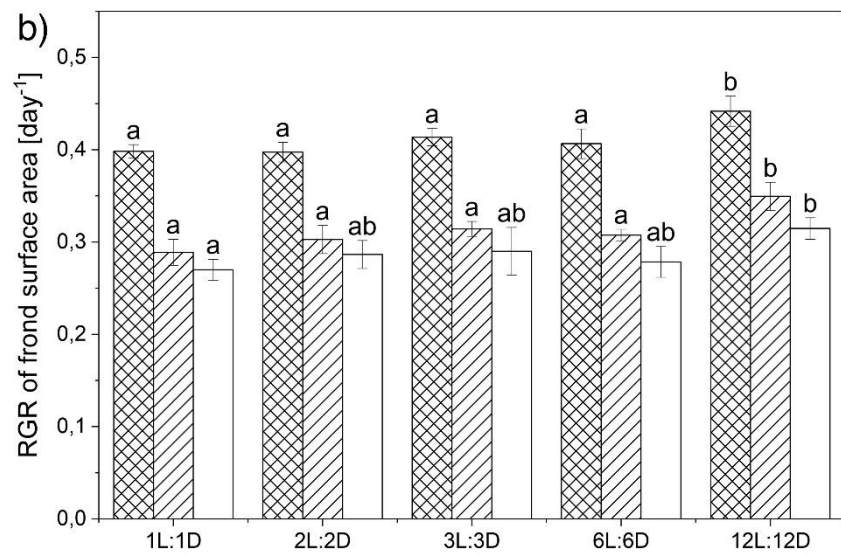
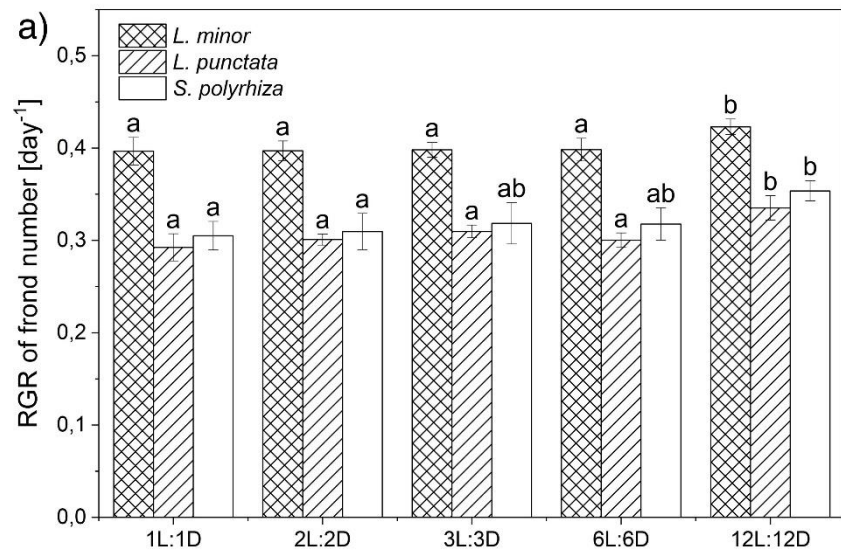


Figure 4. RGR values calculated based on (a) frond number and (b) frond surface area, together with (c) dry biomass of *L. minor*, *L. punctata*, and *S. polyrhiza* under different intermittent photoperiod regimes (1L:1D, 2L:2D, 3L:3D, 6L:6D, 12L:12D). Values represent means $\pm$ SD ($n = 4$). Different letters indicate significant differences among photoperiod regimes within each species (Tukey HSD; Games–Howell for surface area-based RGR of *S. polyrhiza*, $p < 0.05$).

Nitrogen removal remained relatively stable across photoperiod variants ($46.89 \pm 5.31 - 52.64 \pm 8.40\%$ for *L. minor*, $38.67 \pm 2.88 - 42.63 \pm 3.92\%$ for *L. punctata*, and $54.57 \pm 4.88 - 63.37 \pm 2.51\%$ for *S. polyrhiza*), with no significant differences ($p > 0.05$), potentially because the high initial nitrogen concentration diminished regime-related effects. By contrast, phosphorus removal was higher overall and more sensitive to photoperiod structure, particularly in *L. minor* and *L. punctata*, where less fragmented cycles generally resulted in higher removal than more fragmented regimes (1L:1D, 2L:2D, 3L:3D) (Fig. 5b). In *L. minor*, phosphorus removal ranged from 72.64 ± 2.51 (2L:2D) to $81.46 \pm 3.49\%$ (6L:6D) and in *L. punctata* from 51.40 ± 5.94 (1L:1D) to $75.52 \pm 3.53\%$ (12L:12D). In *S. polyrhiza*, the same tendency was observed but remained non-significant ($p = 0.2048$), consistent with its limited RGR variation, and the removal ranged from 84.09 ± 3.08 to $94.91 \pm 2.67\%$.

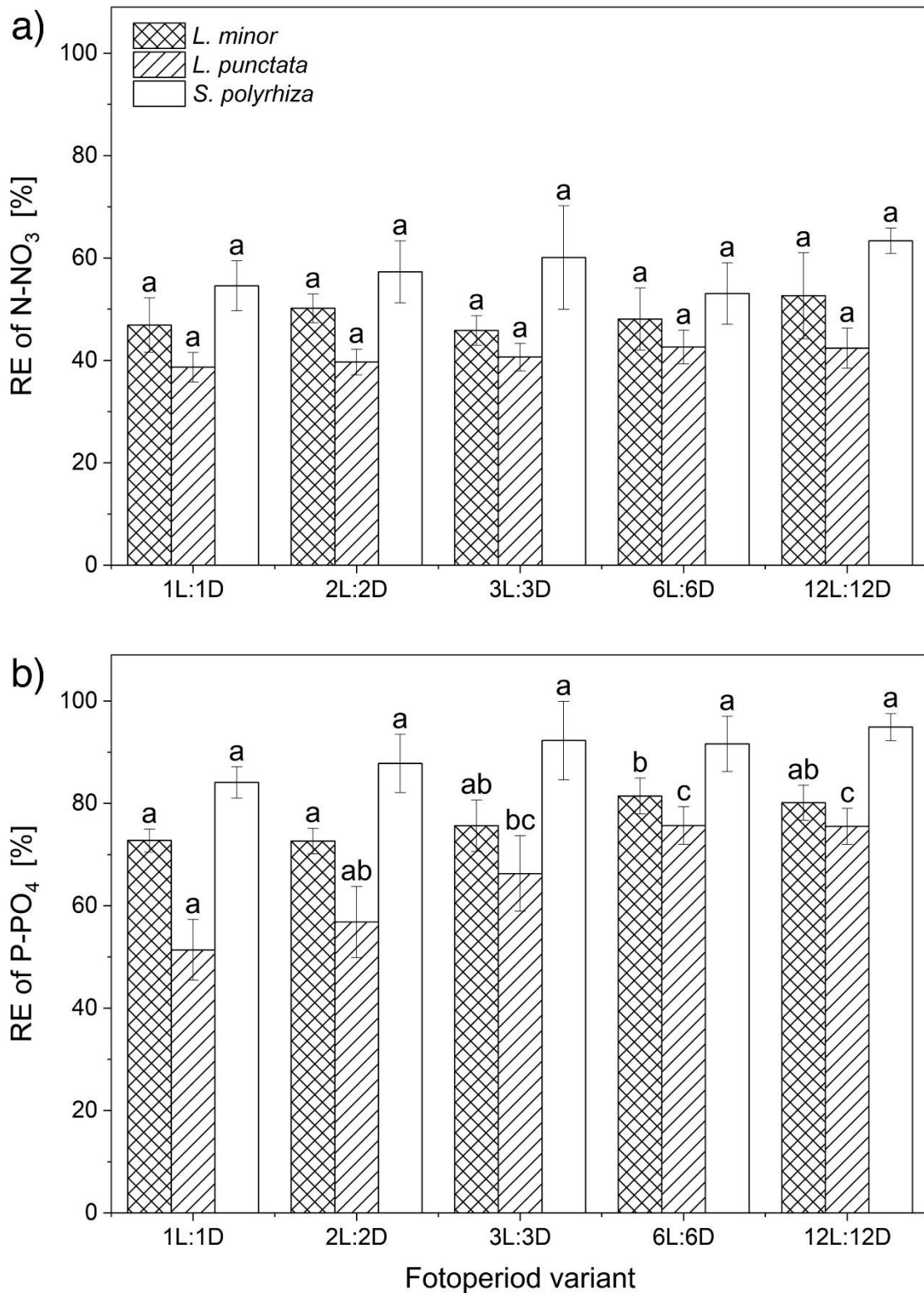
Intermittent, also referred to as fragmented, cyclic, or skeleton photoperiod regimes, involve dividing the photoperiod into multiple shorter light and dark cycles while maintaining the same DLI. Such modifications of photoperiod structure may influence plant growth and physiology, even when the total light dose remains constant, as they can interfere with the plant's internal timekeeping system, known as the circadian clock [42]. This mechanism enables organisms to anticipate and prepare for day–night changes. In plants, it responds to environmental cues, such as light and temperature and, in turn regulates gene expression, phytohormone signaling, and metabolic pathways, thus coordinating key physiological processes including photosynthesis,

respiration, carbon fixation, and contributing to the regulation of morphogenesis and flowering [43]. A mismatch between circadian rhythms and external light–dark signals may modify plants growth responses under multiple light–dark cycles.

For example, Chen and Yang (2018) [44] demonstrated that certain fragmented photoperiod regimes, compared with the standard 16L:8D cycle, stimulated shoot biomass accumulation in lettuce. On the contrary, Hang et al. (2019) [45] showed that increasing photoperiod fragmentation reduced lettuce growth, resulting in lower biomass accumulation, fewer leaves, and reduced leaf area (compared to 12L:12D photoperiod). Similarly, García-Caparrós et al. (2020) [46] reported that increasing the number of light–dark cycles led to declines in chlorophyll content, total soluble sugars, and proline concentrations in tomato and cucumber. Highkin and Hanson (1954) [47] showed that both shorter (6L:6D) and longer (24L:24D) cycles, compared with the standard 12L:12D regime, resulted in significantly reduced growth in tomato. At the same time, the effect was less substantial in pea and absent in sunflower, indicating a species-specific response to photoperiod structure. In this context, the absence of significant differences in *S. polyrhiza* growth, apart from the 1L:1D regime, may suggest that this species is less sensitive to photoperiod fragmentation than *L. minor* and *L. punctata*. However, no comparable studies on duckweed have been reported to date.

Photoperiod fragmentation may induce changes in the expression of circadian clock and photosynthesis-related genes, accompanied by alterations in pigment content, photosynthetic efficiency, and stomatal regulation [48, 49]. Similar mechanisms may have occurred in duckweed in the present study, potentially contributing to reduced growth and nutrient uptake under fragmented photoperiod regimes. An important aspect of the present study is that the daily light integral (DLI) was maintained constant ($\sim 12.96 \text{ mol m}^{-2} \text{ d}^{-1}$) across all spectral and photoperiod treatments. This experimental design allowed us to isolate the effects of light quality and photoperiod structure from total light dose, which is often a confounding factor in

403 plant-light interaction studies. Despite identical DLI, fragmented photoperiod regimes
404 generally resulted in reduced growth, and, in some cases, lower nutrient removal compared to
405 the standard 12L:12D cycle. This suggests that duckweed performance is not solely governed
406 by cumulative photon input but is strongly influenced by the temporal distribution of light,
407 likely through interactions with circadian regulation and photosynthetic acclimation processes.
408 These findings support the concept that optimizing lighting strategies in duckweed-based
409 cultivation and wastewater treatment systems should consider not only DLI but also the
410 continuity and structure of the photoperiod.



411

412 **Figure 5.** RE of (a) N-NO₃ (b) P-PO₄ of *L. minor*, *L. punctata*, and *S. polyrhiza* under different
 413 intermittent photoperiod regimes (1L:1D, 2L:2D, 3L:3D, 6L:6D, 12L:12D). Values represent
 414 means $\pm$ SD (n = 4). Different letters indicate significant differences among photoperiod
 415 regimes within each species (Tukey HSD, p < 0.05).

CONCLUSIONS

This study provides a comprehensive evaluation of how light intensity, spectral composition, and photoperiod structure jointly regulate duckweed growth and nutrient removal under controlled conditions. By maintaining a constant daily light integral across spectral and photoperiod treatments, we demonstrated that duckweed performance is influenced not only by total light dose but also by the temporal distribution and spectral quality of radiation. Growth responses showed clear saturation patterns with increasing light intensity, while dry biomass accumulation continued to increase beyond the RGR saturation range, indicating endpoint-dependent light responses.

Spectral composition effects were species-specific; however, red-enriched white light consistently supported higher growth rates and nutrient removal efficiencies, suggesting its potential suitability for optimized indoor cultivation and water treatment applications. In contrast, fragmented photoperiod regimes generally reduced growth and, in some cases, phosphorus removal, despite identical DLI, highlighting the physiological importance of continuous light–dark cycles and potential circadian regulation mechanisms.

From an applied perspective, these findings indicate that the optimization of duckweed-based cultivation and wastewater treatment systems should consider not only light intensity but also spectral composition and photoperiod structure to maximize light-use efficiency and process performance. The results further suggest that increasing light dose beyond the saturation range may not proportionally enhance growth, emphasizing the need for energy-efficient lighting strategies in controlled environment systems. Future studies should verify whether the observed light responses persist under real wastewater conditions and across different nutrient regimes and duckweed clones to improve the transferability of laboratory findings to large-scale applications.

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585

586

587 **Authors contributions**

588 P.C.: methodology; investigation; data curation; formal analysis; visualization; writing –
589 original draft. J.K.: conceptualization; methodology; writing – review and editing; supervision;
590 funding acquisition

591 **Competing interests**

592 The authors declare no competing interests.

593 **Data availability**

594 The datasets generated during and/or analysed during the current study are available from the
595 corresponding author on reasonable request

596 **Ethical Approval**

597 All authors have read, understood, and have complied as applicable with the statement on
598 "Ethical responsibilities of Authors" as found in the Instructions for Authors”

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