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Acclimation of Charophytes to Spectral Composition of Light at Limiting Irradiances

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ABSTRACT

Irradiance is regarded as a key factor determining the maximum colonization depth (Z_0) of submerged macrophytes. However, the relevance of acclimation capabilities to spectral composition under such low-light conditions remains poorly understood. To address this gap, the present study tested whether spectral composition, in addition to light intensity, affects physiological performance in *Chara globularis* Thuill. and *Chara hispida* L., two charophyte species differing in depth distribution. Individuals were cultivated for 5 weeks under three low irradiances below the photosynthesis light saturation level (6, 12, and 24 µmol photons $m^{-2}s^{-1}$); each irradiance given at four different spectral compositions (full spectrum and blue-, green-, or yellow-wavelength dominated). Daily growth rate, biomass-related parameters, pigment composition (chlorophylls and carotenoids), and photosynthetic light-response parameters were quantified. Across treatments, growth declined with decreasing irradiance in both species, confirming light quantity as the primary constraint, whereas spectral composition had no significant effect on growth or biomass-related parameters. Pigment composition differed consistently between species, with *C. globularis* exhibiting higher chlorophyll *a* and carotenoid concentrations across all treatments. Spectral effects on pigment composition were weak, restricted to the highest irradiance, and observed exclusively in *C. globularis*. Despite these differences, photosynthetic parameters remained largely stable across treatments. The initial slope of the photosynthesis-irradiance curve (α) showed no interspecific differences, while the light saturation point (I_k) was consistently higher in *C. globularis* than in *C. hispida*. Overall, physiological responses were dominated by light intensity and species identity, while spectral effects were minor and conditional. These findings indicate that charophytes, owing to their comparatively low organizational complexity and maintenance demands, do not appear to gain a functional advantage from strong spectral acclimation under low irradiance. Instead, physiological stability emerges as the prevailing strategy, with the greater plasticity of *C. globularis* potentially facilitating persistence near the lower macrophyte limit.

1 | Introduction

Beside nutrient availability, physical factors such as temperature and light are key elements structuring macrophyte communities in aquatic ecosystems (Barko et al. 1982; Jeppesen et al. 2000). Along depth gradients, both light intensity and spectral composition change as light propagates through the water column (Wetzel 2001; Kauer et al. 2010). These depth-related intensity and spectral shifts arise from the combined effects of wavelength-specific absorption and scattering processes, which together determine light attenuation (Bamber 1986). Absorption mostly comes from the water itself, particle-bound and dissolved pigments (Thrane et al. 2014), while scattering is dependent from particle properties (Bohren et al. 2008), with a strong wavelength dependence from particle size (Peng and Effler 2011). Increased nutrient availability, especially phosphate enrichment, has been shown to enhance

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phytoplankton biomass and consequently pigment absorption whereas coloured dissolved organic matter (CDOM) mainly arises from terrestrial sources (Cole 2009; Karlsson et al. 2009). Collectively, phytoplankton and CDOM modify the underwater light climate through the absorption of photosynthetically active radiation (PAR), particularly at short wavelengths (Monteith et al. 2007; Nelson and Siegel 2013). Consequently, both light intensity and spectral composition undergo changes with depth, increasingly constraining the light environment of benthic macrophytes (Churilova et al. 2020).

Light availability at the sediment surface is further modulated by biological and biogeochemical processes operating at both the pelagic and benthic level. At the ecosystem scale, eutrophication-driven phytoplankton blooms can substantially reduce water transparency, triggering regime shifts from clear-water, macrophyte-dominated states to turbid, phytoplankton-dominated states (Scheffer et al. 1993; Jeppesen et al. 2000). At the benthic scale, periphyton accumulation on macrophyte surfaces represents an additional and often underappreciated source of light attenuation, which can suppress macrophyte growth independently of water column transparency (Tóth and Palmer 2016). In charophytes specifically, carbonate encrustation of the thallus surface constitutes a further source of self-shading, as the deposition of $CaCO_3$ during photosynthesis reduces the photon flux reaching the underlying tissue (Beilby and Casanova 2014). Together, these processes compound the depth-related decline in irradiance and collectively constrain the maximum colonization depth (Z_0) of submerged macrophytes, defined as the lowest limit for the growth of macrophytes that either adhere to the sediment or root in it (EN 15460). While light quantity and spectral composition undergo changes with depth, there is a lack of data concerning the physiological and/or morphological acclimation of freshwater macrophytes under extreme low-light conditions. Freshwater macrophytes predominantly rely on chlorophyll *b*-type antennae, including populations occurring at Z_0 e.g., Chl-*b* type antenna in Charophytes (Schubert, Bernhardt, et al. 2024), which restricts their acclimation options compared to marine red algae, having spectrally flexible phycobilin-antenna systems (Gantt et al. 2003; Matthijs et al. 1999).

Charophytes, classified as streptophytes (Schubert, Bernhardt, et al. 2024), frequently dominate the deepest macrophyte stands in clear lakes and play a pivotal role in structuring benthic vegetation patterns (Middelboe and Markager 1997). Within the Charophytes, a variety of species exhibit distinct habitat preferences within the depth gradient of a lake. For instance, *Chara hispida* L. generally establishes dense colonies in shallow, well-lit environments (Blindow et al. 2024), while *Chara globularis* Thuill. has been observed to occur across the entire water column (Zviedre and Schubert 2024) and frequently attains the Z_0 (e.g., lake Garda 17.5 m; Bolpagni et al. 2013). As demonstrated in earlier experimental studies, charophytes have been shown to exhibit plastic responses to light (Schneider et al. 2015; Küster et al. 2005). Furthermore, interspecific differences in responses to light intensity have been demonstrated within Characeae (Küster et al. 2004; Maidel and Schubert 2026). Species-specific wavelength optima have also been reported for vascular macrophytes, such as two *Myriophyllum* species (Lin et al. 2025), suggesting that spectral acclimation may differ among taxa. However, the majority of prior experimental research on charophytes has centred on variations in white-light intensity, with limited examination of the spectral composition of light. Consequently, the combined effects of light intensity and spectral composition under low-light conditions remain insufficiently understood, particularly with respect to species-specific acclimation strategies.

In order to fill this gap of knowledge, acclimation of two Charophyte species to low irradiances mimicking the spectral composition expected close to Z_0 in different types of water bodies was investigated. The initial hypothesis posited that the two species are able to perform spectra-specific acclimation of pigmentation, resulting in optimized photosynthesis and, consequently growth. Specifically, it was hypothesized that *C. globularis*, in accordance with its more extensive ecological distribution, would demonstrate a higher acclimation capacity in response to light and spectral treatments when compared with *C. hispida*. The alternative would be that charophytes, owing to their structurally simpler organization compared with higher vascular macrophytes (Schubert, Holzhausen, et al. 2024), possess lower metabolic and maintenance requirements, and therefore may not have evolved specific spectral acclimation mechanisms at irradiances close to energy limitation.

2 | Materials and Methods

2.1 | Cultivation Conditions

Chara hispida individuals were taken at the first of April 2025 near Langhagen (Mecklenburg-Vorpommern, Germany, N 53° 40′ 34.323″ E 12° 26′ 13.998″), while *C. globularis* individuals were collected the same day in the Peene Valley (N 53° 52′ 16.639″ E 13° 47′ 36.326″). The Langhagen site is a small artificial standing water body, classified as an excavated lake, where habitat water had an oxygen saturation of 94.17% ± 0.45% (means ± SD) and a pH of 6.96 ± 0.21. The Peene Valley site is located within a river-associated wetland landscape along the Peene, where habitat water had an oxygen saturation of 129.94% ± 1.10% and a pH of 7.70 ± 0.33. Water temperature during measurements ranged from 7.67°C to 8.00°C at Langhagen and from 11.03°C to 11.17°C in the Peene Valley. At both sites individuals were taken by a rake approximately 5 m from the shoreline, corresponding to sampling depths of 1.5 m for *C. globularis* and 2 m for *C. hispida*. Habitat water and sediment collected from the respective sampling sites. After sampling, the material was transferred to the laboratory in cooling boxes at ambient temperature and identified. Field identification was confirmed using a stereomicroscope SZX16 (Olympus Corporation, Tokyo, Japan) by means of the determination key of van de Weyer et al. (2024). The key distinguishes the species mainly by the development of stipulodes and cortication. In *C. globularis*, the stipulodes are papillose, whereas in *C. hispida* they are elongated, pointed, and arranged in two rows. In addition, *C. hispida* possesses spine cells that clearly protrude from the cortex.

For cultivation, 0.6 L glass vessels (Weck Glass and Packaging GmbH Bonn, Germany) were used. Cultivation took place over a period of 5 weeks in the climate-controlled 20°C greenhouse of the Institute of Biosciences at the University of Rostock (Mecklenburg-Vorpommern, Germany, N 54° 4′ 36.455″ E 12° 6′ 44.564″) from the first of April 2025

onwards. For cultivation, approximately 100 mL of the specific habitat sediment was placed in a glass vessel without prior sterilisation and covered with 400 mL of the corresponding habitat water. The upper axes (the apical 4 cm) of the organisms were then planted in the sediment, ensuring that there were at least three whorls of the axis above sediment. Three individuals were planted in each cultivation vessel, with six independent vessels per treatment combination. The vessels were then transferred into different cultivation conditions. For this study, three light intensities (approx. 6, 12 and 24 μmol photons m^{-2} s^{-1}) were achieved by adding layers of black gauze with a mesh width of 1 mm. Intensities were adjusted at a cloudy day at noon (12 a.m.–13 p.m.) with a light meter LI-250A (LI-COR Inc. Lincoln, Nebraska, USA). For each intensity four different spectra (full spectra, green, yellow, blue) were achieved by means of coloured foils (Figure 2; green: Lee Nr. 101, Lee Green; blue: Lee Nr. 172 Lagoon blue and yellow: Lee Nr. 101, Yellow Lighting, all obtained from Lee Filters Worldwide; Central Way, Walworth Business Park, Hampshire, UK). The respective foil was placed on top of cultivation containers with blackened sides; each containing four cultivation vessels (Figure 1). All containers were evenly distributed throughout the greenhouse to minimize cross-shadowing at low solar elevation. Individuals were maintained under greenhouse (natural light) conditions, which varied seasonally in intensity and day length. Figure 1 gives an overview about the experimental process and design from sampling in habitat water bodies to analysis.

Absorption spectra of six individuals per species were measured immediately after field sampling using a spectrophotometer (Specord 9.1, VEB Carl Zeiss JENA, DDR-2037, Jena, Germany) equipped with an integrating sphere. Out of these spectra, percentage of absorbed irradiance was calculated for the different experimental light treatments (full spectrum, green-, yellow-, and blue- wavelength dominated light; Figure 2). For each treatment, the spectrum recorded at the highest available light intensity was used for subsequent analyses. All spectra were restricted to the photosynthetically active radiation range (400–700 nm) and normalised by trapezoidal integration to a common total photon flux corresponding to 100% incident light. The resulting normalised spectra were used as reference incident light spectra to calculate spectrally absorbed photon flux (Figure S1), with results shown in Figure 3.

2.2 | Growth and Biomass Determination

Growth was measured at individual A (Figure 1) photographically every 4–7 days using a Sony Alpha 7 camera (Sony Corporation, Tokyo, Japan). Image analysis was performed using the software ImageJ (Version 1.54g, U.S. National Institutes of Health, Bethesda, Maryland, USA) where the total length of each individual was measured from the base to the top of the last whorl to calculate the daily growth rate (DGR) in cm per day. Plant length was recorded after a 5-day pre-acclimation phase, following Nowak et al. (2018), and this measurement was used as the initial reference value. Thereafter, plant length was measured at 5- to 6-day intervals until the end of the experiment, and DGR was calculated for each interval.

After the 5-week cultivation period, biomass analyses were conducted. Individual B (Figure 1) was removed from the cultivation vessel and gently blotted dry for Fresh Mass (FM) determination. To determine the Dry Mass (DM), the samples were dried in a drying oven (Model 600, Memmert GmbH + Co. KG, Schwabach, Germany) at 100°C for 4 h. Subsequently, samples were combusted at 400°C for 1 h in a muffle furnace (Model LVT 154H1-N1, Nabertherm GmbH, Germany) and weighed again to determine Ash Mass (AM). Combustion loss was calculated as DM-AM (ash-free DM). Throughout the manuscript, AM refers to the residual Ash Mass obtained under this specific combustion protocol. This modified loss-on-ignition (LOI) approach was used to obtain relative estimates of combustion loss and residual mineral fraction within the

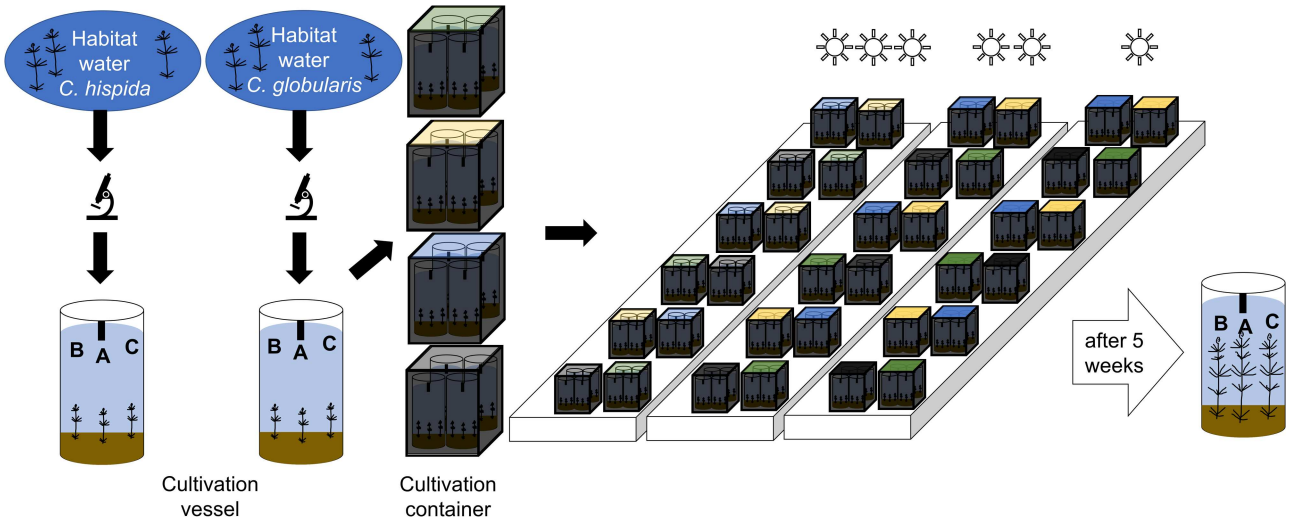


FIGURE 1 | Overview of the experimental process and design from sampling in habitat water bodies to analysis. Cultivation took place in three light intensities (approx. 24, 12 and 6 μmol photons $m^{-2}s^{-1}$) and four spectra (green-, blue-, yellow- wavelength dominated light and full spectrum). Individual A was used for growth documentation, individual B for biomass determination and Individual C was used for photosynthetic and pigment determination.

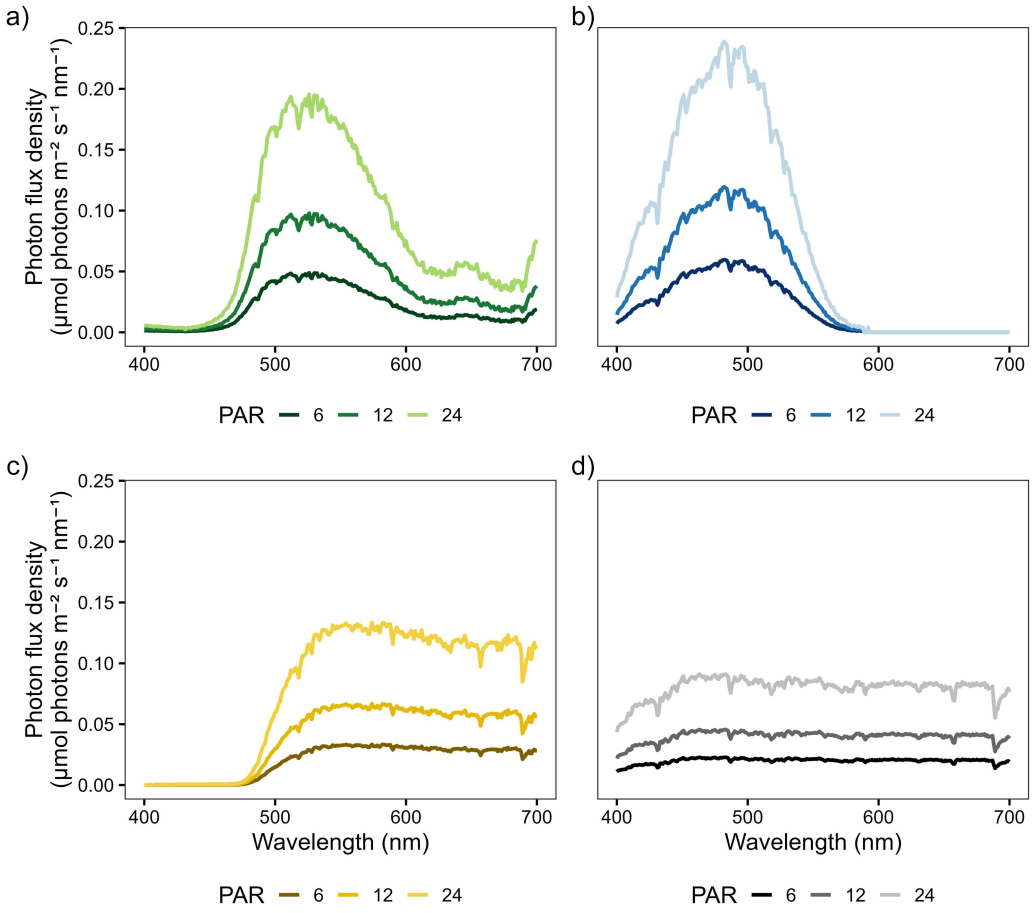


FIGURE 2 | Light conditions used for cultivation in the experiment. (a) Green-, (b) blue-, (c) yellow- wavelength domninated light and (d) full spectra from the wavelength 400–700 nm with light intensities of 6, 12 and 24 µmol photons $m^{-2}s^{-1}$.

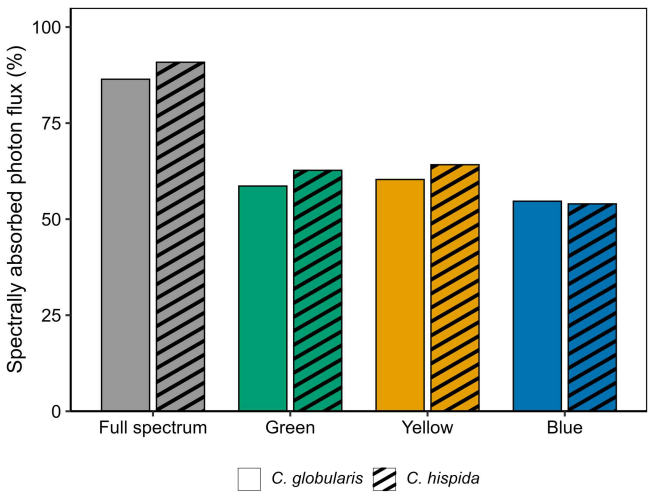


FIGURE 3 | Spectrally absorbed photon flux of *Chara globularis* (solid bars) and *Chara hispida* (hatched bars) under the different experimental light spectra. Bars show the proportion of incident spectral photon flux (400–700 nm) that can theoretically be absorbed by each species under full spectrum, green-, yellow-, and blue-wavelength dominated light conditions. Values were calculated as the spectral overlap between normalised incident light spectra and species-specific mean absorption spectra (Figure S1) measured from field-collected material prior to cultivation.

experimental design, rather than as a standard multi-step LOI analysis. At the lowest light intensity (6 µmol photons $m^{-2}s^{-1}$), all *C. hispida* individuals died before the end of the 5-week cultivation period. Biomass, pigment, and photosynthetic analyses could therefore not be conducted for this species at this treatment level.

2.3 | Pigment Analyses

Pigment analysis were measured at Individual C (Figure 1). The organisms were blotted dry, weighed and transferred into 15 mL centrifuge tubes. Each tube was filled with 6 mL N,N-dimethylformamide (DMF, Carl Roth GmbH, Karlsruhe, Germany) and incubated in darkness for 24 h at 5°C. After incubation, the supernatant was transferred into a 5 cm quartz cuvette and absorbance spectra were measured with a photometer (DR3900, Version 1.1, Hach Lange GmbH, Düsseldorf, Germany). Samples were diluted with DMF if necessary. Pigment concentrations of chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), and carotenoids (Car) were calculated according to Wellburn (1994).

2.4 | Photosynthetic Analyses

Photosynthesis parameters were measured at Individual C (Figure 1) using a pulse-amplitude-modulated (PAM)

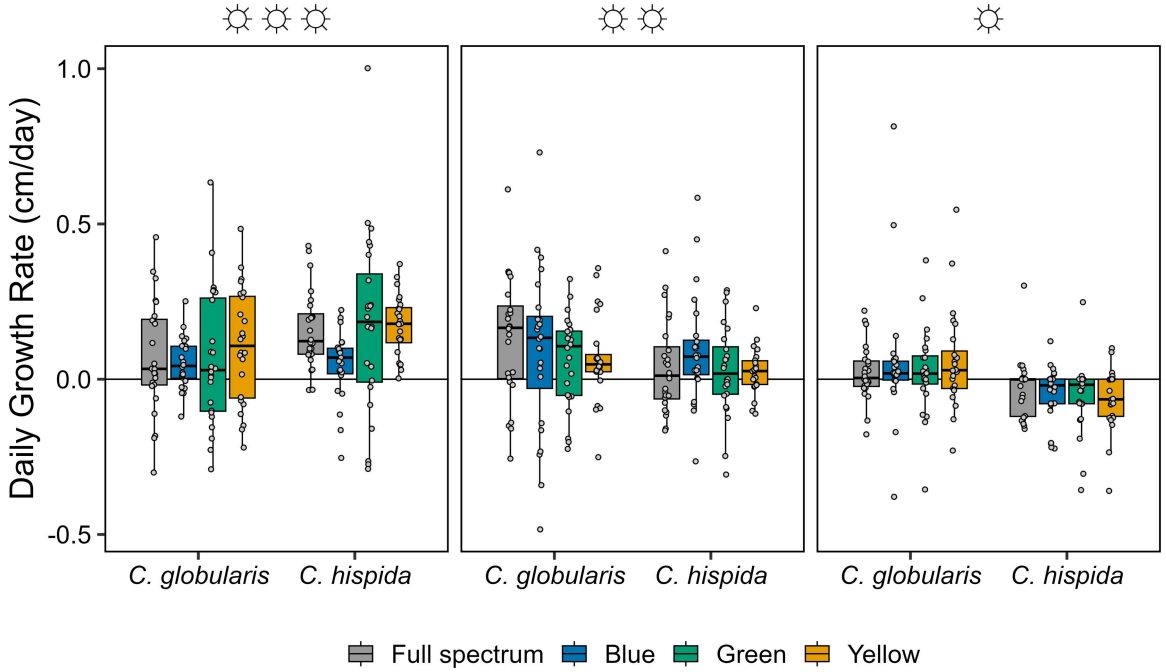


FIGURE 4 | Light intensity and light quality effects on the daily growth rate (DGR) in *Chara globularis* and *Chara hispida* under different light intensities (24 = left, 12 = middle, 6 = right, μmol photons $m^{-2}s^{-1}$) and light spectra (full spectra, blue-, green- and yellow-wavelength-dominated). Data are given as box-whisker plots with interquartile ranges (IQR, boxes) including medians (horizontal lines), whiskers (extending to the most extreme data points with a maximum 1.5 times the interquartile ranges) and raw data points (grey dots). Within light and spectra conditions no significant differences were recorded (LMM/Tukey, $p > 0.05$).

chlorophyll fluorometer (DUAL-PAM-100, Heinz Walz GmbH, Effeltrich, Germany). Before each measurement, the cultivation vessels were dark-adapted for 30 min. Subsequently, the apical parts of the thalli consisting of approximately three whorls were placed into a 1 mL quartz cuvette filled with respective habitat water. Photosynthesis-irradiance (PI) curves were generated using sequential light intensities of 6, 15, 28, 52, 78, 107, 149, 203, 272, 357, 455, 569, 722, 894, 1110, 1723, and 3242 μmol photons $m^{-2}s^{-1}$. The first four irradiance steps lasted 30 s each, and subsequent steps 50 s each. Data were recorded with the Dual-PAM software (Version 3.21, Heinz Walz GmbH). Additional parameters such as the initial slope (α) and the light-saturation point (I_k), were calculated by fitting the PI-curves to the model of Eilers and Peeters (1988) using Microsoft Excel Solver (Microsoft Office LTSC Professional Plus, 2021).

2.5 | Data Analysis

Unless otherwise stated, statistical analyses and graphical visualizations were performed in R version 4.3.1 (R Foundation for Statistical Computing, Vienna, Austria). All data were transformed with logarithmic transformation (base 10) to approximate normally distribution. Normally distribution was tested using Shapiro–Wilk test and homogeneity of variances was tested using Levene's test, implemented in the package *car* (Fox et al. 2012). If assumptions of normality and homoscedasticity were met, one-way analysis of variance (ANOVA) was conducted to test for differences among treatments. Post hoc comparisons following ANOVA were performed using Tukey's Honest Significant Difference (HSD) test via the package *emmeans* (Lenth 2023).

Graphical representation of the results was performed using the package *ggplot2* (Wickham 2016). Multi-panel figure layouts were created with *patchwork* (Pedersen 2025). To indicate statistically significant group differences, compact letter codes were generated using the package *multcompView* (Graves and Piepho 2015). For direct pairwise comparisons involving two experimental groups, significance levels were additionally visualized using the *ggsignif* package (Ahlmann-Eltze and Patil 2019).

Daily growth rate (DGR; cm/day) was analysed using a linear mixed-effects model (LMM) implemented in *lme4*. Species, spectral treatment (full spectrum, blue-, green-, yellow-wavelength dominated light) and light intensity (24, 12, 6 μmol photons $m^{-2}s^{-1}$) and their interactions were included as fixed effects, while plant identity was fitted as a random intercept to account for repeated measurements. Post-hoc comparisons of estimated marginal means were conducted using Tukey-adjusted pairwise contrasts (*emmeans*), and group differences were visualised with compact letter displays (*multcompView*). Model assumptions were assessed using residual diagnostics.

3 | Results

3.1 | Growth and Biomass

The daily growth rate (DGR) of both *C. hispida* and *C. globularis* exhibited a trend of a positive correlation with light intensity, with the highest values recorded under the most intense light treatment and a progressive decline in DGR towards lower light levels (Figure 4). Across all intensity levels, *C. globularis*

exhibited consistently higher DGRs compared to *C. hispida*, with the latter reaching negative values at the lowest light intensity. However, this phenomenon is merely a trend, and it does not exert a significant effect on DGR (LMM/Tukey post hoc tests, $p > 0.05$).

The ratio of fresh mass (FM) to combustion loss dry mass (Ash-free DM) in *C. globularis* and *C. hispida* (Figure 5a) was generally not significantly affected by light intensity or light spectrum within either species. An exception to this pattern was observed in *C. globularis*, where the FM/Ash-free DM ratio exhibited higher levels under medium light conditions at full spectrum and yellow-wavelength-dominated light compared with the corresponding spectral treatments under lower light (Figure 5a, middle vs. right). When comparing spectra between species, *C. globularis* exhibited a significantly lower FM/Ash-free DM ratio under blue-wavelength-dominated light than *C. hispida* under blue and green-wavelength-dominated at the highest light intensity (Figure 5a, left). Furthermore, under medium light conditions, *C. globularis* exhibited a lower ratio under

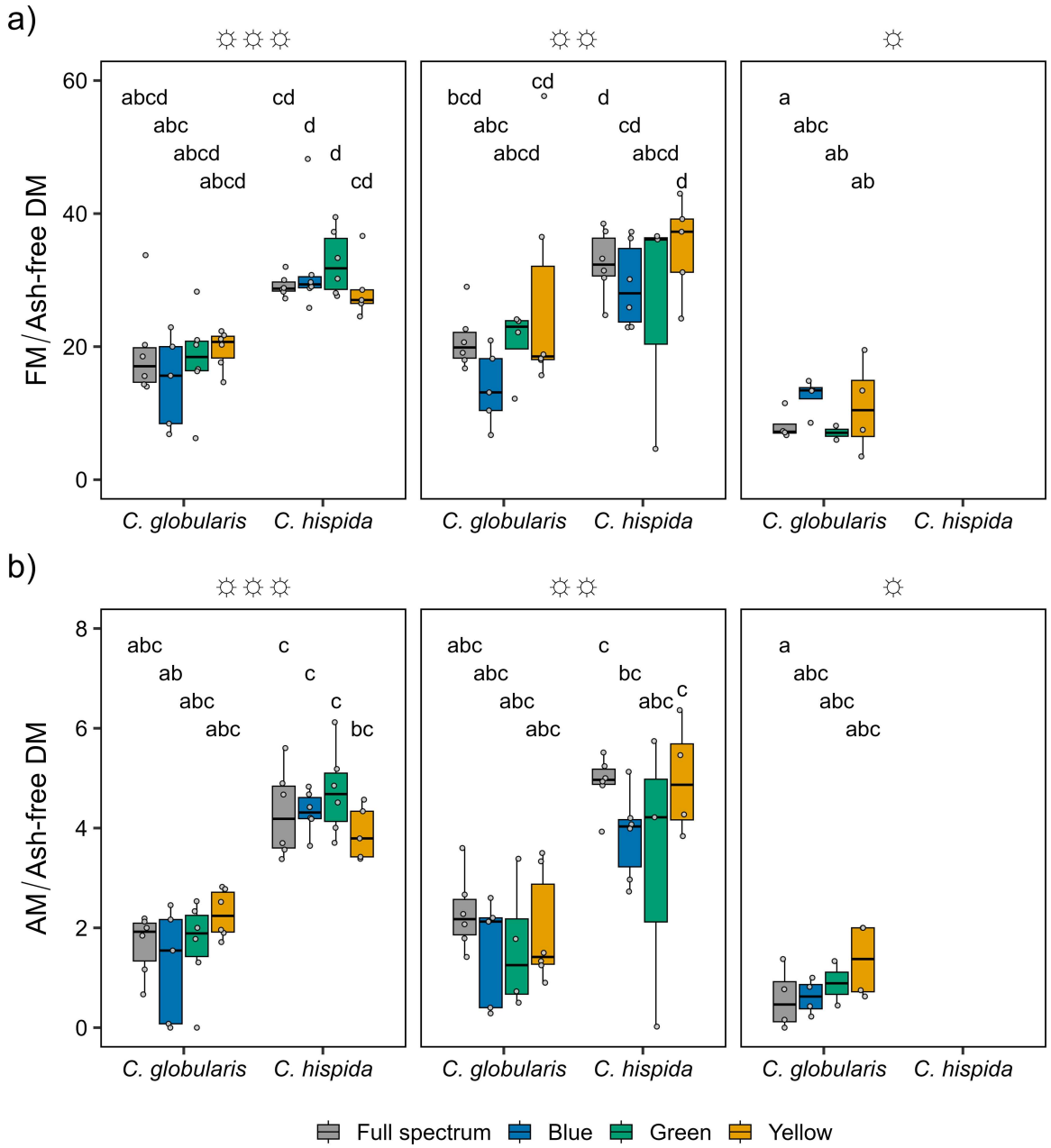


FIGURE 5 | Light intensity and light quality effects on (a) the ratio of fresh mass (FM) to combustion loss dry mass - ash mass (DM-AM) and (b) AM to combustion loss DM of the species *Chara globularis* and *Chara hispida* after 5 weeks of cultivation. Light intensities (24 = left, 12 = middle, 6 = right, µmol photons $m^{-2}s^{-1}$) and light spectra (full spectra, blue-, green- and yellow-wavelength-dominated) were used. $N = 4–6$ individuals per experimental condition. Data are given as box-whisker plots with interquartile ranges (IQR, boxes) including medians (horizontal lines), whiskers (extending to the most extreme data points with a maximum 1.5 times the interquartile ranges) and raw data points (grey dots). Within light and spectra conditions equal letters connect homogeneous mean groups (ANOVA/Tukey, $p < 0.05$). No endpoint data were available for *C. hispida* under 6 µmol photons $m^{-2}s^{-1}$ due to mortality before final measurements.

blue-wavelength-dominated in comparison with *C. hispida* under full spectrum and yellow-wavelength-dominated light at equivalent light intensity (Figure 5a, middle).

The AM/ash-free DM ratio exhibited no substantial effects of light intensity in either species. Conversely, within a species and light-intensity level, no significant effects of light spectrum were detected. A between-species comparison at the highest light intensity (Figure 5b, left) revealed that *C. globularis* under blue-wavelength-dominated exhibited a significantly lower ratio than *C. hispida* under full-spectrum, blue-, and green-wavelength-dominated light treatments. No significant spectral differences between the two species were observed under medium light intensity (Figure 5b, middle).

3.2 | Pigmentation

The Chl *a* and Car content exhibited no substantial reaction to light intensity within either species (Figure 6a,b all panels). The light spectrum exerted an influence on Chl *a* and Car solely at the maximum light intensity, where *C. globularis* cultivated under blue-wavelength-dominated light exhibited higher Chl *a* and Car concentrations than plants cultivated under yellow-wavelength-dominated light (Figure 6a,b, left). When comparing species, *C. globularis* consistently exhibited significantly higher concentrations of Chl *a* and Car than *C. hispida* across all light intensities. No significant spectral effects were detected at lower light intensities.

The Chl *a* + Chl *b*/Car ratio exhibited no substantial effects of light intensity in either species (Figure 6c, all panels). Conversely, the light spectrum influenced the ratio within *C. globularis* at the highest light intensity. Plants cultivated under yellow-wavelength-dominated light exhibited a significantly higher Chl *a* + Chl *b*/Car ratio compared with plants cultivated under blue-wavelength-dominated light (Figure 6c, left). This effect was not observed at the lower light intensities or for *C. hispida*.

3.3 | Photosynthesis

In *C. globularis* exposed to green-wavelength-dominated treatment, the light-saturation point (I_k) of the photosynthesis-irradiance (PI) curve exhibited a significant increase at the highest intensity compared to the lowest light intensity (Figure 7, left vs. right). Across all light intensities, *C. hispida* exhibited lower I_k values than *C. globularis*; however, this difference was not statistically significant. Within the context of a particular species, no substantial effects of light spectrum on I_k were observed under any given light condition. Conversely, a between-species comparison at the maximum light intensity exhibited a substantial discrepancy with *C. globularis* demonstrated a significantly higher I_k than *C. hispida* under the full-spectrum treatment (Figure 7, left).

4 | Discussion

The light intensities employed in this study correspond to conditions near the maximum colonization depth (Z_0), where submerged macrophytes typically experience less than 10% of the surface irradiance (Middelboe and Markager 1997; Kirk 2010). Accordingly, the daily growth rates of *C. globularis* and *C. hispida* in this study decreased with decreasing irradiance. No signs of compensatory elongation were observed, as reported by Maidel and Schubert (2026) for *Chara virgata* for low light intensities (18 μmol photons $m^{-2}s^{-1}$). At such low light conditions as in this study, growth limitation can be caused by restricted availability of energy for carbon gain and downstream metabolism (Gao et al. 2023). In contrast to light intensity, light quality did not affect daily growth rates across treatments. This is noteworthy, as earlier studies on other algal taxa, such as *Chlorella* spp. (Harder and Bederke 1957) and *Chlamydomonas* spp. (Brown and Geen 1974), reported substantially lower growth rates under green light compared to blue and red light. In line with the observed growth rates, biomass-related parameters followed a similar pattern (Figure 5, Figures S2 and S3). Species-specific differences were evident: *C. hispida* increased biomass without elongation growth, whereas *C. globularis* showed no differences. While *C. hispida* exhibited higher biomass ratios, *C. globularis* showed no significant biomass response despite lower elongation rates, indicating a decoupling of elongation and biomass accumulation. This pattern may reflect species-specific differences in thallus architecture, cortication, and residual mineral fraction. Within species, biomass-related parameters showed little sensitivity to both, light intensity and spectral composition (Figure 5).

Also, the pigment composition differs strongly between species, with *C. globularis* consistently exhibiting higher chlorophyll *a* and carotenoid concentrations than *C. hispida* across all light intensities (Figure 6). This pattern is consistent with the broader ecological amplitude of *C. globularis* and indicates greater investment in light-harvesting and photoprotective pigments, characteristic of macrophytes occupying wider depth ranges and low-light habitats (Middelboe and Markager 1997; Schubert, Bernhardt, et al. 2024; Maidel and Schubert 2026). Spectral effects on pigment composition were restricted to the highest irradiance and occurred only in *C. globularis*. With respect to pigment composition, it is known that blue-enriched light stimulates carotenoid synthesis (Samuolienė et al. 2017) and play a role in photoprotection (Shropshire 1980) while yellow-enriched light shows substantially lower absorption by photosynthetic pigments. In this study increased carotenoids as well as chlorophyll *a* concentration relative to yellow-wavelength dominated light support this pattern. The Chl($a + b$)/Car ratio showed an opposing pattern (Figure 6) which can be interpreted as indicative of an imbalance between the absorbed energy and photochemical capacity (Samuolienė et al. 2017; Shropshire 1980). However, because the present study assessed endpoint responses after 5 weeks, short-term physiological adjustments such as state-transition-like regulation, which can balance excitation energy between PSII and PSI on a timescale of minutes (Bellafiore et al. 2005), were not assessed and cannot be excluded. Therefore, the limited pigment response observed here should be interpreted as a lack of pronounced long-term pigment-based acclimation under the tested conditions. Whether Characeae are capable of state transitions remains, to our knowledge, unresolved and would require targeted short-term fluorescence-based measurements.

The light-saturation point (I_k) showed only marginal sensitivity to light intensity and spectral composition, consistent with

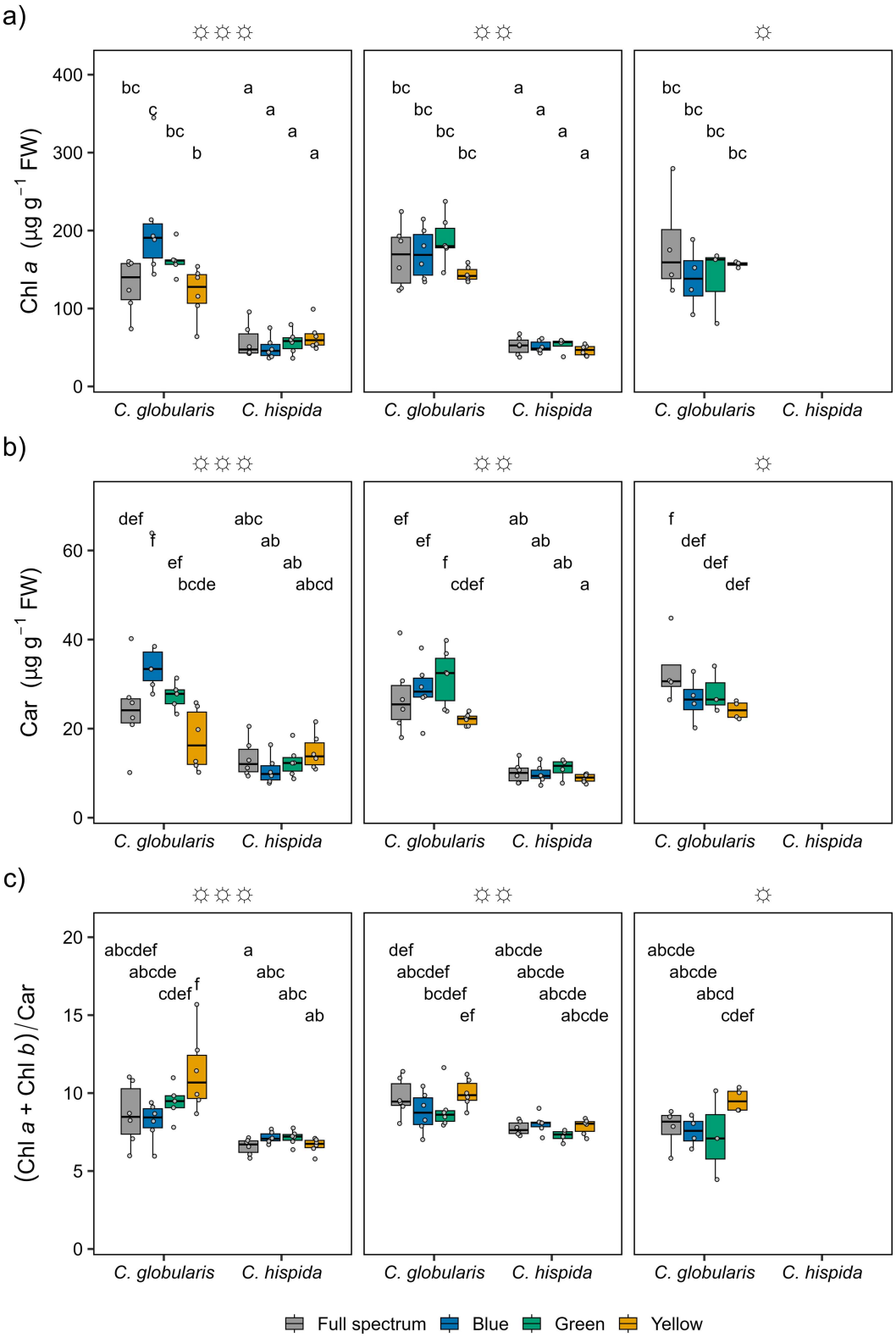


FIGURE 6 | Light intensity and light quality effects on (a) chlorophyll a (Chl a), (b) carotenoids (Car) and (c) sum of Chl *a* and chlorophyll *b* (Chl *b*) divided to Car concentration of *Chara globularis* and *Chara hispida* after 5 weeks of cultivation. Light intensities (24 = left, 12 = middle, 6 = right, µmol photons $m^{-2}s^{-1}$) and light spectra (full spectra, blue-, green- and yellow-wavelength-dominated) were used. $N = 3–6$ individuals per experimental condition. Data are given as box-whisker plots with interquartile ranges (IQR, boxes) including medians (horizontal lines), whiskers (extending to the most extreme data points with a maximum 1.5 times the interquartile ranges) and raw data points (grey dots). Within light and spectra conditions equal letters connect homogeneous mean groups (ANOVA/Tukey, $p < 0.05$). No endpoint data were available for *C. hispida* under 6 µmol photons $m^{-2}s^{-1}$ due to mortality before final measurements.

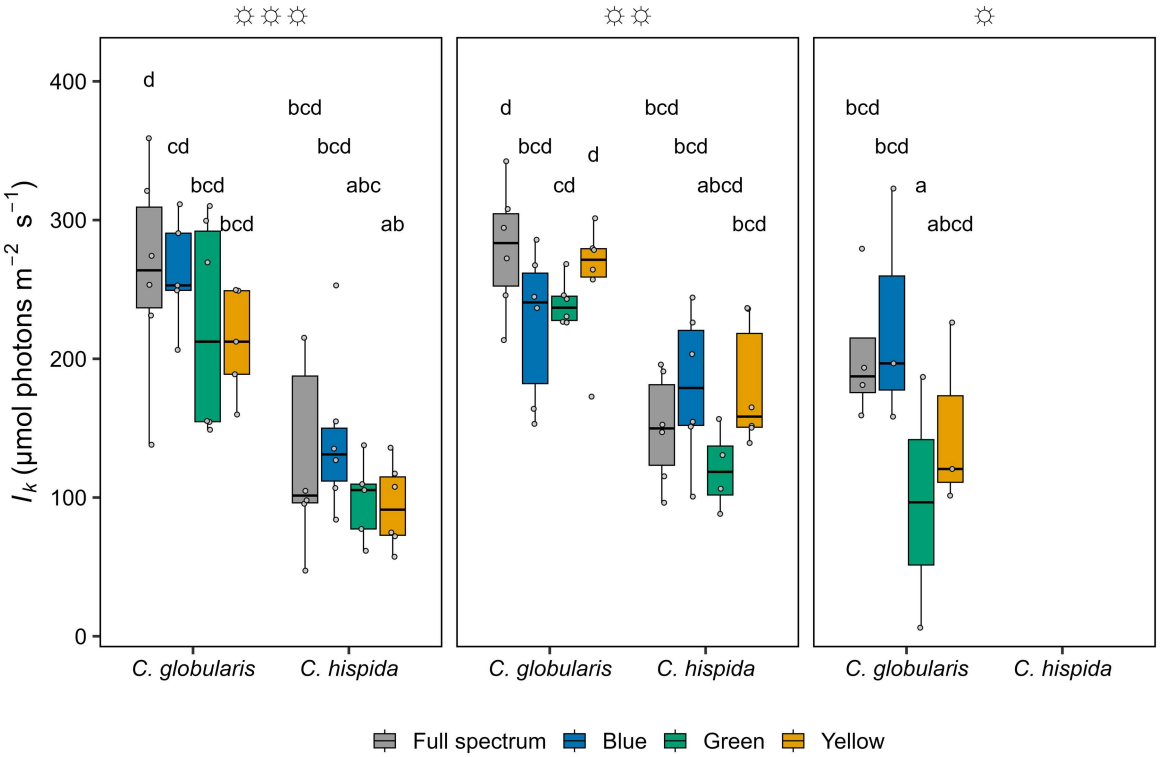


FIGURE 7 | Light intensity and light quality effects on the light saturation point (I_k) of the photosynthesis irradiation (PI) curve of *Chara globularis* and *Chara hispida* after 5 weeks of cultivation. Light intensities (24 = left, 12 = middle, 6 = right, µmol photons $m^{-2}s^{-1}$) and light spectra (full spectra, blue-, green- and yellow-wavelength-dominated) were used. $N = 3–6$ individuals per experimental condition. Data are given as box-whisker plots with interquartile ranges (IQR, boxes) including medians (horizontal lines), whiskers (extending to the most extreme data points with a maximum 1.5 times the interquartile ranges) and raw data points (grey dots). Within light and spectra conditions equal letters connect homogeneous mean groups (ANOVA/Tukey, $p < 0.05$). No endpoint data were available for *C. hispida* under 6 µmol photons $m^{-2}s^{-1}$ due to mortality before final measurements.

previous reports of stable I_k values across growth irradiances in charophytes (Küster et al. 2004; Schneider et al. 2015). A stable I_k can result from the interaction of multiple photosynthetic parameters, particularly the maximal electron transport rate (ETR_{max}) and α (Figure S4). In this study, α remained largely constant across treatments and showed only minor increases at the highest light intensity in *C. hispida* under yellow and green light (Figure S4). This limited responsiveness contrasts with pronounced spectral acclimation of light-harvesting and energy-transfer processes reported for other green algae, such as *Chlamydomonas reinhardtii* and *Chlorella variabilis* (Ueno et al. 2019). A likely explanation for this discrepancy is the substantially higher irradiance used by Ueno et al. (2019; 50 µmol photons $m^{-2}s^{-1}$ under continuous illumination), compared with the lower irradiances applied in the present study. Thus, the light intensities used here may have remained below the threshold required to induce strong spectral acclimation, resulting in only minor treatment effects on α, ETR_{max} and consequently I_k.

However, this interpretation should be restricted to the two species and experimental conditions tested here, as charophyte responses to environmental stressors can be species- and ecotype-specific. For example, Rojo et al. (2019) showed that the effects of ultraviolet (UV) radiation, warming, and nitrate enrichment on growth, morphology, pigments, and photoprotective compounds differed between *Chara* species and between populations from different habitat types. Beyond interspecific variation within Characeae, a comparison with vascular macrophytes further contextualises these findings: *Potamogeton perfoliatus* has been shown to adjust both morphological and photophysiological traits along spectral gradients within the water column (Tóth and Palmer 2016), and chlorophyll *a*:*b* ratios in floating-leaved macrophytes respond to spectral light quality changes associated with lake browning (Rajala et al. 2024). The absence of comparable spectral plasticity in the present study is consistent with the structural and physiological differences between charophytes and vascular aquatic plants (Schubert, Holzhausen, et al. 2024).

The low spectral flexibility observed here supports the hypothesis that charophytes do not rely on, nor appear to benefit from strong spectral acclimation under the tested low-light conditions. Instead, persistence at low irradiance appears to depend on stable physiological performance under reduced energy availability, favouring functional stability over plasticity. This physiological stability should not be interpreted as an absence of regulatory capacity, but rather as an energetically rational response to severe photon limitation. Acclimation of light-harvesting architecture can occur in different ways. In vascular macrophytes, it is reflected in the structural differentiation of sun and shade leaves. This is a committed form of acclimation because it requires de novo leaf construction. In cyanobacteria and red algae, comparable adjustment occurs through phycobilisome-based complementary chromatic

adaptation (Gantt et al. 2003; Matthijs et al. 1999). Such acclimation carries biosynthetic costs. These costs may not be recoverable when energy input itself is the primary constraint. In charophytes, with their comparatively simple thallus organisation and low biomass-specific maintenance demands (Schubert, Holzhausen, et al. 2024), the energetic threshold for profitable acclimation may simply not be reached at the irradiances tested here. When the photosynthetic gain from spectral remodelling is marginal, as suggested by the modest differences in spectrally absorbed photon flux among treatments (Figure 3), maintaining a stable physiological baseline becomes the more adaptive strategy. From an evolutionary perspective, this pattern may reflect selection for persistence under chronic energy limitation rather than for acclimation capacity per se, consistent with the broader observation that organisationally simpler photoautotrophs tend to dominate under extreme photon limitation. This interpretation conceptually aligns with observations from cave light gradients, where a decline in irradiance is associated with a shift toward phototrophic organisms of lower organizational complexity. From our results it can be speculated that vascular plants are restricted to well-lit entrance zones, bryophytes extend further inward, and algae dominate under extreme photon limitation (Pentecost and Zhaohui 2001; Ren et al. 2021).

Notably, despite clear differences in spectral composition (Figure 2), the estimated spectrally absorbed photon flux differed only modestly among treatments and between species (Figure 3), supporting the conclusion that photon supply rather than wavelength composition constrained performance at these irradiances. These data are primarily relevant for very low irradiances such as those occurring at Z_0 and are consistent with the early view proposed by Oltmanns (1892) that light intensity is more important than light quality. This concept has since been supported and discussed in numerous studies (Dring 1981; Dring 1990 Ramus 1981; Ramus 1983; Kirk 1994). Across different light intensities, algae have adapted through a range of distinct mechanisms e.g., by pigment packaging effects. These effects are arising from self-shading of light-absorbing pigments within cells and tissues, which can enhance light absorption under low irradiance or reduce internal light gradients (Osborne and Raven 1986; Brugnoli and Björkman 1992; Terashima and Hikosaka 1995; Kirk 1994; Evans et al. 2004). While brown algae and higher plants can exploit such mechanisms through pigment mobility and chloroplast redistribution (Osborne and Raven 1986), this was rejected for Charophytes (Beilby and Casanova 2014). Persistence of the two charophyte species near Z_0, therefore possibly depends primarily on maintaining a positive carbon balance under photon limitation rather than on adaptive spectral optimisation, reflecting a survival strategy rather than active physiological acclimation.

5 | Conclusions

In summary, our results demonstrate that near the maximum colonization depth (Z_0), charophyte performance is governed primarily by light intensity rather than spectral composition, with species-specific differences in growth and biomass-related traits. Growth and physiological responses indicate limited spectral acclimation, suggesting that under strong photon limitation additional adaptive adjustments are unlikely to be beneficial due to insufficient energy availability. Persistence under such conditions therefore appears to rely on maintaining a positive carbon balance, consistent with light quantity being the dominant constraint on photosynthetic performance near Z_0, independent of species-specific acclimation capacity.

Author Contributions

Alena-Maria Maidel: conceptualization, investigation, writing – original draft preparation. **Vanessa Fabienne Bischoff:** investigation, writing – review and editing. **Hendrik Schubert:** conceptualization, writing – review and editing, supervision. All authors have read and agreed to the published version of the manuscript for publication.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

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

References

Ahlmann-Eltze, C., and I. Patil. 2019. "ggsignif: Significance Brackets for 'ggplot2'" In *R Package Version 0.6. 0*.

Bamber, J. C. 1986. "Attenuation and Absorption." *Physical Principles of Medical Ultrasonics* 2: 93–166.

Barko, J. W., D. G. Hardin, and M. S. Matthews. 1982. "Growth and Morphology of Submersed Freshwater Macrophytes in Relation to Light and Temperature." *Canadian Journal of Botany* 60, no. 6: 877–887. https://doi.org/10.1139/b82-113.

Beilby, M. J., and M. T. Casanova. 2014. *The Physiology of Characean Cells*. Springer.

Bellafiore, S., F. Barneche, G. Peltier, and J. D. Rochaix. 2005. "State Transitions and Light Adaptation Require Chloroplast Thylakoid Protein Kinase STN7." *Nature* 433, no. 7028: 892–895.

Blindow, I., R. Romanov, H. Schubert, N. Stewart, and K. van de Weyer. 2024. "Chara Hispida." In *Charophytes of Europe*, 527–541. Springer.

Bohren, Craig F., and D. R. Huffman. 2008. *Absorption and Scattering of Light by Small Particles*. John Wiley & Sons.

Bolpagni, R., E. Bettoni, F. Bonomi, et al. 2013. "Charophytes of the Lake Garda (Northern Italy): A Preliminary Assessment of Diversity and Distribution." *Journal of Limnology* 72, no. 2: 31.

Brown, T. J., and G. H. Geen. 1974. "The Effect of Light Quality on the Carbon Metabolism and Extracellular Release of Chlamydomonas Reinhardtii Dangeard[1]." *Journal of Phycology* 10, no. 2: 213–220.

Brugnoli, E., and O. Björkman. 1992. "Chloroplast Movements in Leaves: Influence on Chlorophyll Fluorescence and Measurements of Light-Induced Absorbance Changes Related to ΔpH and Zeaxanthin Formation." *Photosynthesis Research* 32, no. 1: 23–35.

Churilova, T. Y., V. V. Suslin, N. A. Moiseeva, and T. V. Efimova. 2020. "Phytoplankton Bloom and Photosynthetically Active Radiation in Coastal Waters." *Journal of Applied Spectroscopy* 86, no. 6: 1084–1091. https://doi.org/10.1007/s10812-020-00944-0.

Cole, J. J. 2009. "Production in Pristine Lakes." *Nature* 460, no. 7254: 463–464.

Dring, M. J. 1981. "Chromatic Adaptation of Photosynthesis in Benthic Marine Algae: An Examination of Its Ecological Significance Using a Theoretical Model." *Limnology and Oceanography* 26, no. 2: 271–284.

Dring, M. J. 1990. "Light Harvesting and Pigment Composition in Marine Phytoplankton and Macroalgae." *Light and Life in the Sea 1*: 89–103.

Eilers, P. H. C., and J. C. H. Peeters. 1988. "A Model for the Relationship Between Light Intensity and the Rate of Photosynthesis in Phytoplankton." *Ecological Modelling* 42, no. 3–4: 199–215.

Evans, J. R., T. C. Vogelmann, W. E. Williams, and H. L. Gorton. 2004. "Chloroplast to Leaf." In *Photosynthetic Adaptation: Chloroplast to Landscape. New York, NY*, edited by K. S. William, C. V. Thomas and C. Christa, 15–41. Springer.

Fox, J., S. Weisberg, and D. Adler, et al. 2012. *Package 'Car'*. R Foundation for Statistical Computing, 333.

Gantt, E., B. Grabowski, and F. X. Cunningham. 2003. "Antenna Systems of Red Algae: Phycobilisomes With Photosystem II and Chlorophyll Complexes With Photosystem I." *Advances in Photosynthesis and Respiration* 13: 307–322.

Gao, J., W. Hu, J. Wang, Y. Cui, and L. Li. 2023. "Response of Growth-Related Traits of Submerged Macrophytes to Light Reduction: A Meta-Analysis." *Sustainability* 15, no. 7: 5918. https://doi.org/10.3390/su15075918.

Graves, S., H.-P. Piepho, and M. L. Selzer. 2015. Package 'multcompView' In *Visualizations of Paired Comparisons* 451.

Harder, R., and B. Bederke. 1957. "Über Wachstumsversuche Mit Rot- Und Grünalgen (*Porphyridium Cruentum, Trailliella intricata, Chlorella pyrenoidosa*) in Verschiedenfarbigem, Energiegleichen Licht." *Archiv für Mikrobiologie* 28, no. 2: 153–172.

Jeppesen, E., J. Peder Jensen, M. SØndergaard, T. Lauridsen, and F. Landkildehus. 2000. "Trophic Structure, Species Richness and Biodiversity in Danish Lakes: Changes Along a Phosphorus Gradient." *Freshwater Biology* 45, no. 2: 201–218. https://doi.org/10.1046/j.1365-2427.2000.00675.x.

Karlsson, J., P. Byström, J. Ask, P. Ask, L. Persson, and M. Jansson. 2009. "Light Limitation of Nutrient-Poor Lake Ecosystems." *Nature* 460, no. 7254: 506–509.

Kauer, T., H. Arst, and L. Tuvikene. 2010. "Underwater Light Field and Spectral Distribution of Attenuation Depth in Inland and Coastal Waters." *Oceanologia* 52, no. 2: 155–170.

Kirk, J. T. O. 1994. *Light and Photosynthesis in Aquatic Ecosystems*. Cambridge University Press.

Kirk, J. T. O. 2010. *Light and Photosynthesis in Aquatic Ecosystems*. Cambridge University Press.

Küster, A., R. Schaible, and H. Schubert. 2004. "Light Acclimation of Photosynthesis in Three Charophyte Species." *Aquatic Botany* 79, no. 2: 111–124. https://doi.org/10.1016/j.aquabot.2004.01.010.

Küster, A., R. Schaible, and H. Schubert. 2005. "Sex-Specific Light Acclimation of Chara Canescens (Charophyta)." *Aquatic Botany* 83, no. 2: 129–140. https://doi.org/10.1016/j.aquabot.2005.05.009.

Lenth, R. 2023. emmeans: Estimated Marginal Means, aka Least-Squares Means_. In *R package version 1.8. 5*.

Lin, X., X. Wu, Y. Liu, et al. 2025. "Underwater Light Quality Selection of Invasive and Native *Myriophyllum* Species in China: Invasive Species Prefer Blue Light, While Native Species Prefer Red Light." *Global Ecology and Conservation* 62: e03678. https://doi.org/10.1016/j.gecco.2025.e03678.

Maidel, A. M., and H. Schubert. 2026. "Morphological and Physiological Traits of Two Closely Related Charophyte Species: *Chara virgata* Kütz. and *C. Globularis* Thuill." *Aquatic Botany* 202: 103929. https://doi.org/10.1016/j.aquabot.2025.103929.

Matthijs, H. C. P., J. H. Geerdink, and H. Balke, et al. 1999. "Sensing of Green Light in Complementary Chromatic Adaptation of the Cyanobacterium *Calothrix* sp Involvement of a Rhodopsin?" In *The Phototrophic Prokaryotes*, 187–194. Springer.

Middelboe, A. L., and S. Markager. 1997. "Depth Limits and Minimum Light Requirements of Freshwater Macrophytes." *Freshwater Biology* 37, no. 3: 553–568.

Monteith, D. T., J. L. Stoddard, C. D. Evans, et al. 2007. "Dissolved Organic Carbon Trends Resulting From Changes in Atmospheric Deposition Chemistry." *Nature* 450, no. 7169: 537–540. https://doi.org/10.1038/nature06316.

Nelson, N. B., and D. A. Siegel. 2013. "The Global Distribution and Dynamics of Chromophoric Dissolved Organic Matter." *Annual Review of Marine Science* 5, no. 1: 447–476. https://doi.org/10.1146/annurev-marine-120710-100751.

Nowak, P., H. Schubert, A. Holzhausen, V. Sommer, and R. Schaible. 2018. "Morphological Adaptations of *Chara baltica* and *Chara liljebladii* (Characeae) Under Different Light Conditions." *Botany Letters* 165, no. 1: 67–75.

Oltmanns, F. 1892. Ueber die Cultur- und Lebensbedingungen der-Meersalgen. 23: 349–440.

Osborne, B. A., and J. A. Raven. 1986. "Light Absorption by Plants and Its Implications for Photosynthesis." *Biological Reviews* 61, no. 1: 1–60.

Pedersen, T. L. 2025. "Patchwork: The Composer of Plots." R Package Version 1.3.2.9000. https://patchwork.data-imaginist.com.

Peng, F., and S. W. Effler. 2011. "Characterizations of the Light-Scattering Attributes of Mineral Particles in Lake Ontario and the Effects of Whiting." *Journal of Great Lakes Research* 37, no. 4: 672–682. https://doi.org/10.1016/j.jglr.2011.07.002.

Pentecost, A., and Z. Zhaohui. 2001. "The Distribution of Plants in Scoska Cave, North Yorkshire, and Their Relationship to Light Intensity."

Rajala, S., S. Estlander, L. Nurminen, K. Sainio, and J. Horppila. 2024. "Seasonal Fluctuations in Pigment Content of Macrophytes: Implications for Monitoring Brownification." *Hydrobiologia* 851, no. 3: 633–648.

Ramus, J. 1981. "The Capture and Transduction of Light Energy." *Biology of Seaweeds* 17: 458–492.

Ramus, J. 1983. "A Physiological Test of the Theory of Complementary Chromatic Adaptation. II. Brown, Green and Red Seaweeds 1." *Journal of Phycology* 19, no. 2: 173–178.

Ren, H., F. Wang, W. Ye, et al. 2021. "Bryophyte Diversity Is Related to Vascular Plant Diversity and Microhabitat Under Disturbance in Karst Caves." *Ecological Indicators* 120: 106947. https://doi.org/10.1016/j.ecolind.2020.106947.

Rojo, C., E. Puche, and M. A. Rodrigo. 2019. "The Antagonistic Effect of UV Radiation on Warming or Nitrate Enrichment Depends on Ecotypes of Freshwater Macroalgae (Charophytes)." *Journal of Phycology* 55, no. 3: 714–729.

Samuolienė, G., A. Viršilė, A. Brazaitytė, et al. 2017. "Blue Light Dosage Affects Carotenoids and Tocopherols in Microgreens." *Food Chemistry* 228: 50–56.

Scheffer, M., S. H. Hosper, M. L. Meijer, B. Moss, and E. Jeppesen. 1993. "Alternative Equilibria in Shallow Lakes." *Trends in Ecology & Evolution* 8, no. 8: 275–279.

Schneider, S. C., D. E. Pichler, T. Andersen, and A. Melzer. 2015. "Light Acclimation in Submerged Macrophytes: The Roles of Plant Elongation, Pigmentation and Branch Orientation Differ Among Chara Species." *Aquatic Botany* 120: 121–128. https://doi.org/10.1016/j.aquabot.2014.05.002.

Schubert, H., K.-G. Bernhardt, M. T. Casanova, T. Gregor, W.-H. Kusber, and B. Turner. 2024. "Taxonomic Concepts and Taxonomic Evolution in European Characeae." In *Charophytes of Europe*, 65–99. Springer.

Schubert, H., A. Holzhausen, P. Nowak, and M. T. Casanova. 2024. "Ontogenesis and Life Cycle of Charophytes." In *Charophytes of Europe*, edited by I. Hendrik Schubert, E. Blindow, H. Nat, T. Korsch, L. D. Gregor, et al., 13–33. Springer Nature.

Shropshire, W., Jr. 1980. "Carotenoids as Primary Photoreceptors in Blue-Light Responses." In *The Blue Light Syndrome*, 172–186. Springer.

Terashima, I., and K. Hikosaka. 1995. "Comparative Ecophysiology of Leaf and Canopy Photosynthesis." *Plant, Cell & Environment* 18, no. 10: 1111–1128.

Thrane, J. E., D. O. Hessen, and T. Andersen. 2014. "The Absorption of Light in Lakes: Negative Impact of Dissolved Organic Carbon on Primary Productivity." *Ecosystems* 17, no. 6: 1040–1052. https://doi.org/10.1007/s10021-014-9776-2.

Tóth, V. R., and S. C. J. Palmer. 2016. "Acclimation of Potamogeton Perfoliatus L. to Periphyton Accumulation-Induced Spectral Changes in Irradiance." *Hydrobiologia* 766, no. 1: 293–304.

Ueno, Y., S. Aikawa, A. Kondo, and S. Akimoto. 2019. "Adaptation of Light-Harvesting Functions of Unicellular Green Algae to Different Light Qualities." *Photosynthesis Research* 139, no. 1: 145–154.

Wellburn, A. R. 1994. "The Spectral Determination of Chlorophylls a and b, as Well as Total Carotenoids, Using Various Solvents With Spectrophotometers of Different Resolution." *Journal of Plant Physiology* 144, no. 3: 307–313.

Wetzel, R. G. 2001. *Limnology: Lake and River Ecosystems*. Gulf Professional Publishing.

van de Weyer, K., R. Romanov, and I. Blindow. 2024. "Determination Key Characeae." In *Charophytes of Europe*, edited by H. S. Irmgard Blindow, E. N. Heiko Korsch, T. G. Luc Denys, et al., 201–223. Springer Nature.

Wickham, H. 2016. "Getting Started With ggplot2." In *ggplot2: Elegant Graphics for Data Analysis*, 11–31. Springer.

Zviedre, E., and H. Schubert. 2024. "Chara Globularis." In *Charophytes of Europe*, edited by I. B. Hendrik Schubert, H. K. Emile Nat, L. D. Thomas Gregor, et al., 499–508. Springer Nature.

Supporting Information

Additional supporting information can be found online in the Supporting Information section.

Figure S1: Normalised photon flux density (μmol photons nm^{-1}) of the light spectra used in the experiment and the corresponding absorption spectra of *Chara globularis* and *Chara hispida*.

Figure S2: Light intensity and light quality effects on the fresh mass (FM) of the species *Chara globularis* and *Chara hispida* after 5 weeks of cultivation. Light intensities (24 = left, 12 = middle, 6 = right, μmol photons $m^{-2}s^{-1}$) and light spectra (full spectra, blue, green and yellow-wavelength-dominated) were used.

Figure S3: Light intensity and light quality effects on the dry mass (DM) of the species *Chara globularis* and *Chara hispida* after 5 weeks of cultivation.

Figure S4: Light intensity and light quality effects on the initial slope (α) of the photosynthesis irradiation (PI) curve of *Chara globularis* and *Chara hispida* after 5 weeks of cultivation.