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RESEARCH

Optimizing culture conditions for micropropagation of *Codium tomentosum*

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Abstract

Codium tomentosum is currently cultivated through vegetative propagation (cuttings) in tumble culture systems targeting niche markets. To scale up and expand its aquaculture production, optimization of propagation and culture methods is key. In Asia, artificial seed production via regeneration of isolated utricles and medullary filaments has already been established for the commercial production of *Codium fragile*. Our preliminary findings indicate that this approach also holds strong potential for *C. tomentosum*. In this context, the present study aimed to identify optimized culture conditions for the micropropagation of this species. The effects of temperature (12 °C, 16 °C, and 20 °C), light intensity (20, 40, 60 and 100 µmol photons m^{-2} s^{-1}), and light spectra (white, blue, red and green) on the development of filamentous thalli from isolated utricles and medullary filaments were investigated. Our findings show that productivity was primarily influenced by light intensity and spectral composition, with optimal growth observed under intermediate irradiance (60 µmol photons m^{-2} s^{-1}) and either blue or white light. Red or white light, low irradiance (20 µmol photons m^{-2} s^{-1}) and higher temperatures (20 °C), promoted elongation of filamentous thalli. On the other hand, cooler temperatures (12 °C), low irradiance, and exposure to blue or white light enhanced photosynthetic efficiency. Pigment content (Chl *a*, Chl *b*, and total Chl) decreased with increasing irradiance suggesting a light-induced downregulation of pigment synthesis under high-light conditions, while carotenoid levels were only mildly affected by temperature and irradiance. Blue light induced higher pigment accumulation. This study provides important insights into the growth, productivity, and physiological responses of filamentous thalli of *C. tomentosum* under varying abiotic conditions, thereby supporting the development of future nursery protocols based on micropropagation.

Keywords Artificial seed production · Filamentous thalli · F_v/F_m · Seaweed cultivation · Macroalgae · Chlorophyceae

Introduction

The green seaweed *Codium tomentosum* Stackhouse (Chlorophyta, Ulvophyceae, Bryopsidales) is native to the northeast Atlantic and widely distributed along the Portuguese coast (Marques et al. 2021). In recent years, this species has attracted increasing interest due to its potential for product development across various sectors, including food, nutraceuticals, pharmaceuticals, and cosmetics industries. It is consumed as an edible seaweed and has been commercially cultivated for use in gourmet cuisine, due to its mild flavor, soft texture, and rich nutritional profile (Marques et al. 2021; Moreira et al. 2022). Extracts of *C. tomentosum* have shown antioxidant, antimicrobial and anti-inflammatory activities, supporting their potential incorporation in functional food products and dietary supplements (Kalavathy et al. 2025; Sousa et al. 2025). Additionally, bioactive compounds derived from *C. tomentosum*, particularly sulfated polysaccharides, have been explored for use in cosmetic formulations such as moisturizers and skin-protective products due to their hydrating and barrier-enhancing properties (Ashokkumar et al. 2022; Seppic 2024).

Codium tomentosum plays a valuable role in integrated multi-trophic aquaculture (IMTA) systems, where it

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contributes to nutrient recycling and produces high-value biomass for industrial applications (da Costa et al. 2015; Rey et al. 2020; Marques et al. 2021). However, the current scale of production remains limited by currently used propagation methods. Vegetative propagation, used commercially, relies on fragmentation of spongy thalli kept free-floating in tumble culture (Marques et al. 2022). While effective at a small scale, this method is labor-intensive and challenging to scale up. Sexual reproduction, which occurs via the fusion of anisogamous gametes produced in specialized gametangia on the utricles, has been considered as an alternative propagation route for *Codium* species (Borden and Stein 1969; Prince and Trowbridge 2004; Miravalles et al. 2012). However, its commercial application remains underdeveloped due to low seedling establishment success under laboratory conditions (Hwang et al. 2005a). Regarding *C. tomentosum*, the control of its life cycle under laboratory conditions remains a significant challenge, despite recent advances on gametogenesis induction, gamete release, and light requirements for sustained germling growth (Sá et al. 2024).

Micropropagation presents a promising alternative for *Codium* cultivation. This vegetative propagation technique exploits the totipotent capacity of isolated utricles and medullary filaments to regenerate filamentous thalli under controlled conditions (Park and Sohn 1992). Successfully applied in the commercial cultivation of *C. fragile* in Korea (Hwang et al. 2005b; 2008), this method is particularly advantageous as it enables the production of artificial seed stock independent of natural reproductive cycles, while also offering strong potential for biobanking and for seeding substrates intended for sea-based cultivation. Laboratory observations have confirmed the formation of branched, dissociated, coenocytic filamentous thalli from isolated utricles also for *C. tomentosum* (Yang et al. 1997), although the specific culture conditions required to optimize this process remain to be fully characterized.

Codium species exhibit two distinct thallus morphologies: spongy and filamentous. The spongy thallus, the macroscopic adult form commonly observed in natural populations, is composed of a dense peripheral layer of pigmented utricles encasing a central network of colorless medullary filaments, providing both structure and photosynthetic capacity (Borden and Stein 1969; Nanba et al. 2002; 2005). In contrast, the filamentous form consists of fine, branched, siphonous filaments, and can originate from isolated utricles, medullary filaments, or zygotes (Yang et al. 1997; Hwang et al. 2008). This form plays a fundamental role in the early stages of development and vegetative regeneration. The capacity of these isolated cells to regenerate complete thalli reflects a high degree of morphological plasticity and totipotency — an attribute that underpins their use in micropropagation (Park and Sohn 1992). In *C. fragile*, the transition from the filamentous to the spongy morphology is influenced by environmental factors such as water movement, light, nutrients, and temperature, (Park and Sohn 1992; Nanba et al. 2005; González et al. 2014). Comparable transitions are thought to occur in *C. tomentosum*.

This study aims to evaluate the effect of culture conditions, including temperature, light intensity, and spectral quality, on the development of filamentous thalli from isolated utricles of *C. tomentosum*. Filamentous thalli growth, productivity, maximum quantum yield of photosystem II, and pigment composition were assessed. A better understanding of these factors is crucial to optimize micropropagation methods and establish reliable nursery protocols that can support large-scale seedstock production and cultivation, ultimately enhancing the scalability of *C. tomentosum* aquaculture.

Material and methods

Sampling site and acclimation

Specimens of *Codium tomentosum* were randomly collected in northern Portugal during low tide on Viana shore (41°41'48.6"N, 8°51'12.2"W) in November 2024 and Foz-Homem do Leme shore (41°9'36.97"N, 8°41'10.79"W) in February and March 2025. The specimens were transported to the laboratory and washed several times with sterile seawater. Specimens were kept for 24 h until further processing, at 16 °C, 12 h light:12 h dark photoperiod in sterile seawater and aeration, under a light intensity of 100 µmol photons m^{-2} s^{-1}, measured with the US-SQS/L spherical micro quantum sensor connected to a Universal Light Meter ULM-500 (Heinz Walz, Germany).

Isolation of utricles and medullary filaments

Utricles and medullary filaments of *C. tomentosum* were isolated by mechanical fragmentation. A suspension was prepared by homogenizing the spongy thalli (n = 3) in sterile seawater (80 g L^{-1}; Fig. S1 A) using a Multiquick-5 homogenizer (MR 500, Braun GmbH, Germany) at maximum speed for 60 s. Following homogenization, 10 mL aliquots of the resulting suspension were distributed into replicate 55 mm diameter Petri dishes (n = 6) for each tested condition.

Temperature, irradiance and light spectra trials

The effects of temperature, irradiance, and light spectra on the development of filamentous thalli from the isolated utricles and medullary filaments of *C. tomentosum* were investigated across three experimental trials. Trial 1: The effect of three temperatures (12 °C, 16 °C, and 20 °C) was tested under a constant light intensity of 60 µmol photons m^{-2} s^{-1} with a 12 h light:12 h dark photoperiod and white light. The selected temperature range reflects natural conditions across the species distribution, while the light intensity was previously employed for artificial seed production of *C. fragile* (Hwang et al. 2005b). Trial 2: The effect of irradiance was tested at four levels (20, 40, 60 and 100 µmol photons m^{-2} s^{-1}) at 16 °C with a 12 h light:12 h dark photoperiod and white light. The intermediate temperature (16 °C) was chosen as it represents the average sea surface temperature in the main distribution locations of *C. tomentosum* (Gómez-Gesteira et al. 2008). Trial 3: The effect of four different light spectra (white, blue, green and red) was tested at 16 °C using cool white LED lighting provided by Systion Eletronics, Lda (4000 K; 8 × 11 W, Portugal). Red, blue, and green light spectra were obtained by using Lee filters over the light source, with the white light condition left unfiltered. The light spectra were measured using the SpectraPen mini (PSI - Photon Systems Instruments, Czech Republic; Fig. S2). The irradiance was set to 40 µmol photons m^{-2} s^{-1} under a 12 h light:12 h dark photoperiod, as this light intensity provided a balance between productivity and photosynthetic efficiency observed in the previous irradiance trial. All cultures were maintained for 35 days under the respective conditions.

Assessment of biomass productivity and growth

Codium tomentosum biomass was quantified at the beginning and at the end of the trials (n = 6). Fresh biomass was filtered using a vacuum filtration water pump with 50 mm glass microfiber filters, dried at 100 °C for 24 h, and then weighed on a precision scale (model LPG-4202i, VWR International, USA). The productivity was calculated according to the equation:

$$Productivity\left(mg\ DW\ L^{-1}\ day^{-1}\right) = \left(W_f - W_i\right)/(V \times t)$$

where W_f is the final dry weight, W_i is the initial dry weight, V is the volume of culture and t is the time of the experiment in days (Marques et al. 2022)

In the first 21 days, images of filamentous thalli were captured (Fig. S1 B-C) using the Nikon Eclipse TE200 (Japan) connected to a computer with Nikon's microscope imaging software (NIS). The filamentous thalli length (µm) was measured after undergoing post-processing using Image J Software 1.53e (U. S. National Institutes of Health, USA). After 21 days, filamentous thalli became too large to allow accurate measurement of length under the inverted microscope.

Chlorophyll *a* fluorescence

Pulse amplitude modulated (PAM) fluorometry has been used on seaweed culture research to analyze the impacts of culture conditions, such as temperature, photoperiod, light spectra and irradiance, on the photosynthetic activity and physiological condition of cultivated seaweed (Lideman et al. 2013; Tan et al. 2019; Marques et al. 2021; Revilla-Lovano et al. 2021; Marques et al. 2022; Xu et al. 2022). The in vivo chlorophyll *a* fluorescence of photosystem II was assessed at the beginning and end of the experiment using a Junior-PAM fluorometer (Walz, Germany). Each replicate (n = 6), consisting of a suspension of utricles, medullary filaments and filamentous thalli of *C. tomentosum* (Fig. S1 D), was filtered using a vacuum filtration water pump with 50 mm glass microfiber filters. Prior to measurement, samples were dark-adapted for 15 min. F_o (minimal fluorescence after dark adaptation) and F_m (maximal fluorescence after dark adaptation) were measured, with a saturation pulse of 190 µmol photons m^{-2} s^{-1} for 0.5 s to calculate the maximum quantum yield of photosystem II (F_v/F_m). Given that the thin filamentous samples derived from isolated utricles exhibit low optical thickness, PSII saturation is achieved at relatively low irradiance levels; preliminary tests confirmed that this intensity was sufficient to reach maximal Fm, with no further increase observed at higher intensities. The following formula was used (Maxwell and Johnson 2000):

$$F_v/F_m = \left(F_m - F_0\right)/F_m$$

Three saturation pulses (technical replicates) were performed in each biological replicates and the average value was used as an estimate of maximum quantum yield of photosystem II (F_v/F_m) of the biomass.

Photosynthetic pigments analysis

Pigment quantification was performed according to Assunção et al. (2021) and Sousa et al. (2025). Pigment extraction was performed using 100 mg of dried biomass, previously frozen at −80 °C and subsequently freeze-dried (lyophilised). The dried biomass was then mixed with three flat-spoons of 1.4 mm zirconia beads and 3 mL of ethanol:acetone (1:9, v/v). Samples were homogenized using a Precellys Homogenizer (Precellys Evolution, Bertin Technologies, France) with 6 cycles at 8000 rpm for 30 s, each followed by a 40 s pause, and then centrifuged at 4000 rpm for 10 min. The supernatant (1 mL) was transferred to a new vial, and evaporated to dryness under nitrogen flow.

Extracts were resuspended in 1 mL of ethanol (99.9%, v/v) saturated with Na_2CO_3. The following equations were used to determine the content of pigments:

$$\text{Chlorophyll } a\left(\text{Chl a; } \mu\text{g mL}^{-1}\right) = \left(-5.20 \times A_{647}/0.76\right) + \left(13.53 \times A_{664}/0.76\right) \quad (1)$$

$$\text{Chlorophyll } b\left(\text{Chl b; } \mu\text{g mL}^{-1}\right) = \left(22.43 \times A_{647}/0.76\right) - \left(7.07 \times A_{664}/0.76\right) \quad (2)$$

$$\text{Total carotenoids}\left(\mu\text{g mL}^{-1}\right) = 4 \times \left(A_{480} - A_{750}\right) \quad (3)$$

where absorbance (A) was read at λ = 480, λ = 647, λ = 664 and λ = 750 nm. Absorbances were corrected for a 0.76 cm light path (200 µL of sample). Molar extinction coefficient of each pigment was applied according to the solvent used (Lichtenthaler 1987; Ritchie 2006; Connan 2015). All samples were analysed as chemical quadruplicates. The concentration of pigments was expressed as mg per gram of dry weight (mg g^{-1} DW). Total chlorophyll (*a* + *b*) and the chlorophyll *a*/*b* ratio were calculated from the measured chlorophyll concentrations.

Statistical analysis

All results are expressed as mean ± standard deviation (SD). Data distribution was assessed using the Shapiro–Wilk test for normality and Levene's test for homogeneity of variances. When assumptions of normality or homogeneity were met, parametric tests were applied, otherwise, data transformations or alternative statistical approaches were employed. A one-way ANOVA was conducted to evaluate the effect of experimental factors (temperature, irradiance, and light spectra) on productivity and maximum quantum yield of photosystem II (F_v/F_m). A repeated-measures ANOVA was performed to assess the effects of the experimental factors and time on filament length of *C. tomentosum*, with time treated as a within-subject factor and Petri dish considered the experimental unit. When significant main effects or interactions were detected, Post hoc comparisons were performed using estimated marginal means with Tukey adjustment. Additionally, pigment concentrations (chl *a*, chl *b*, and total carotenoids) were analysed as separate response variables within three independent experimental datasets corresponding to temperature, irradiance, and light spectra treatments. Depending on the fulfilment of statistical assumptions, one-way ANOVA was applied for data with normal distribution and homogeneous variances, whereas Welch's ANOVA was used when heteroscedasticity was detected. Significant effects were followed by Tukey's HSD or Games–Howell post hoc tests, as appropriate, to identify pairwise differences among treatments. Spearman correlation was applied to assess the statistical relationship between temperature, irradiance and pigments content (Chl *a*, Chl *b*, Chl (*a* + *b*) and carotenoids) and Chl *a*/*b* ratio. Principal component analysis (PCA) was performed to evaluate the effects of the different light spectra on pigments concentration. Differences were considered statistically significant at $p < 0.05$. Data processing and statistical analyses were performed using R software version 4.5.0, except for the correlation analysis, which was carried out using Prism version 8.0.1.

Results

Temperature

Temperature did not significantly affect the mean productivity of filamentous thalli of *C. tomentosum* ($F_{2,6} = 0.45$, $p = 0.66$; Fig. 1A). Mean productivity values were 8.60 ± 5.57 mg DW L^{-1} day^{-1} at 12 °C, 10.70 ± 3.24 mg DW L^{-1} day^{-1} at 16 °C, and 7.76 ± 2.16 mg DW L^{-1} day^{-1} at 20 °C.

Filamentous thallus growth was strongly time-dependent ($F_{2,30} = 1196.72$, $p < 0.001$), whereas temperature showed no significant main effect ($F_{2,15} = 3.23$, $p = 0.068$). A significant temperature × time interaction was observed ($F_{4,30} = 10.03$, $p < 0.001$; Fig. 1B), indicating treatment-specific temporal growth trajectories. Across all temperatures, thalli length increased sharply over time ($p < 0.001$), with no clear differences among treatments at early stages. Divergence among temperature treatments emerged at later time points, consistent with the significant interaction effect.

Photosynthetic performance, expressed as maximum quantum yield (F_v/F_m), was significantly affected by temperature ($F_{2,15} = 5.44$, $p = 0.017$; Fig. 1C). Cultures at lower temperatures (12 °C), showed higher F_v/F_m (0.55 ± 0.03) compared to those at higher temperatures (20 °C; 0.45 ± 0.05), whereas intermediate (16 °C) did not differ significantly from the other treatments. All temperature treatments exhibited a significant reduction in F_v/F_m relative to initial values.

Pigment concentrations (chl *a*, chl *b*, and total carotenoids) varied significantly with temperature for all pigments analyzed (Chl *a*: $F_{3,28} = 780.6$, $p < 0.001$; Chl *b*: Welch's ANOVA, $F_{3,14.2} = 280.3$, $p < 0.001$; total carotenoids: $F_{3,28} = 64.8$, $p < 0.001$; Fig. 1D). Chl *a* was highest at 20 °C (0.89 ± 0.03 mg g^{-1} DW), while chl *b* peaked at 16 °C (0.50 ± 0.04 mg g^{-1} DW). Carotenoids were lowest at 16 °C (0.21 ± 0.01 mg g^{-1} DW), with similar values at 12 °C and 20 °C ($p > 0.05$). Overall, chl *a*, chl *b,* and carotenoid content decreased from initial values to day 35.

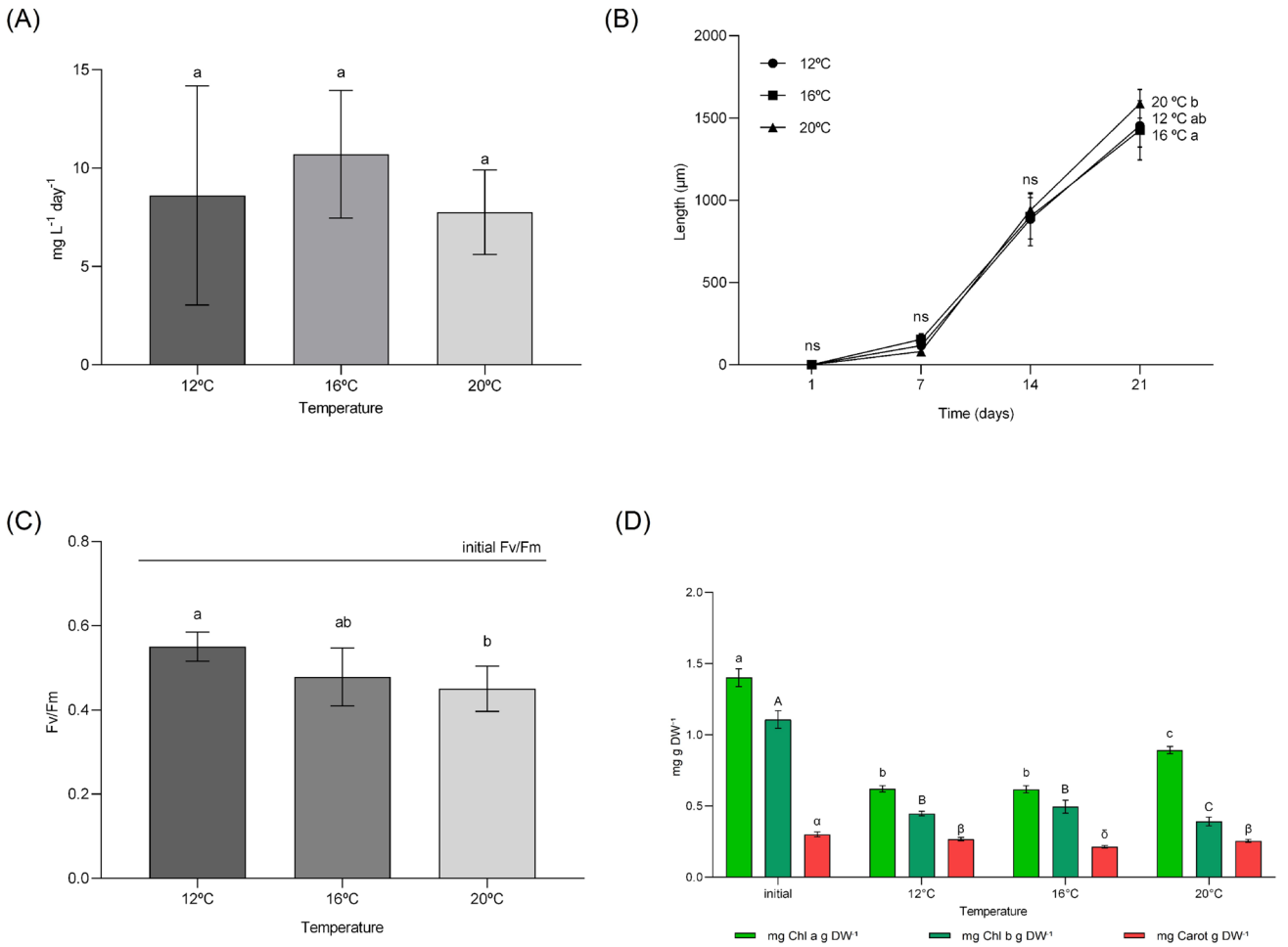


Fig. 1 Effects of temperature (12, 16 and 20 °C) on (A) productivity (mg DW L^{-1} day^{-1}), (B) filamentous thalli length (µm), (C) maximum quantum yield of photosystem II (F_v/F_m), and (D) pigment content (Chl *a*, Chl *b* and carotenoids; mg g^{-1} DW) of *C. tomentosum*. Filamentous thalli developed from isolated utricles and medullary filaments. Cultures were maintained for 35 days (A, C, D) or 21 days (B) under white light (60 µmol photons m^{-2} s^{-1}) and a 12 h light:12 h dark photoperiod. Initial values (day 1) are shown for F_v/F_m and pigments. Different letters indicate significant differences ($p < 0.05$), and ns indicates non-significant differences. For detailed summary data (mean ± SD) and statistical groupings, see Supplementary material (Table S1)

Irradiances

Irradiance had a significant effect on the mean productivity of filamentous thalli of *C. tomentosum* ($F_{3,20} = 4.73$, $p = 0.012$; Fig. 2A). Productivity was highest at 60 µmol photons m^{-2} s^{-1} (46.9 ± 9.1 mg DW L^{-1} day^{-1}), significantly exceeding that at 20 µmol photons m^{-2} s^{-1} (26.0 ± 11.7 mg DW L^{-1} day^{-1}; $p = 0.010$), while no other pairwise differences were detected. Intermediate values were observed at 40 and 100 µmol photons m^{-2} s^{-1} (32.9 ± 8.2 and 31.6± 11.7 mg DW L^{-1} day^{-1}, respectively).

Filamentous thallus length was strongly time-dependent ($F_{2,40} = 708.21$, $p < 0.001$), whereas irradiance had no detectable main effect ($F_{3,20} = 0.24$, $p = 0.867$). A significant irradiance × time interaction was observed ($F_{6,40} = 3.04$, $p = 0.015$; Fig. 2B), indicating treatment-specific temporal trajectories. Across all irradiance levels, thalli length increased sharply over time ($p < 0.001$), while no differences among treatments were evident during early stages. Divergence among irradiance treatments emerged only at later time points, consistent with the interaction effect, with the lowest irradiance (20 µmol photons m^{-2} s^{-1}) resulting in significantly longer filaments (961.9 ± 166 µm) than those at highest irradiances (100 µmol photons m^{-2} s^{-1}; 748.0 ± 104 µm; $p < 0.05$).

Photosynthetic performance (F_v/F_m) was strongly affected by irradiance ($F_{3,20} = 119.6$, $p < 0.001$; Fig. 2C). The highest values were recorded under low irradiance (0.76 ± 0.02 at 20 µmol photons m^{-2} s^{-1}), similar to initial levels (0.79 ± 0.06; $p = 0.082$). A clear and progressive decline in F_v/F_m was observed with increasing irradiance, reaching 0.38 ± 0.04 at 100 µmol photons m^{-2} s^{-1}.

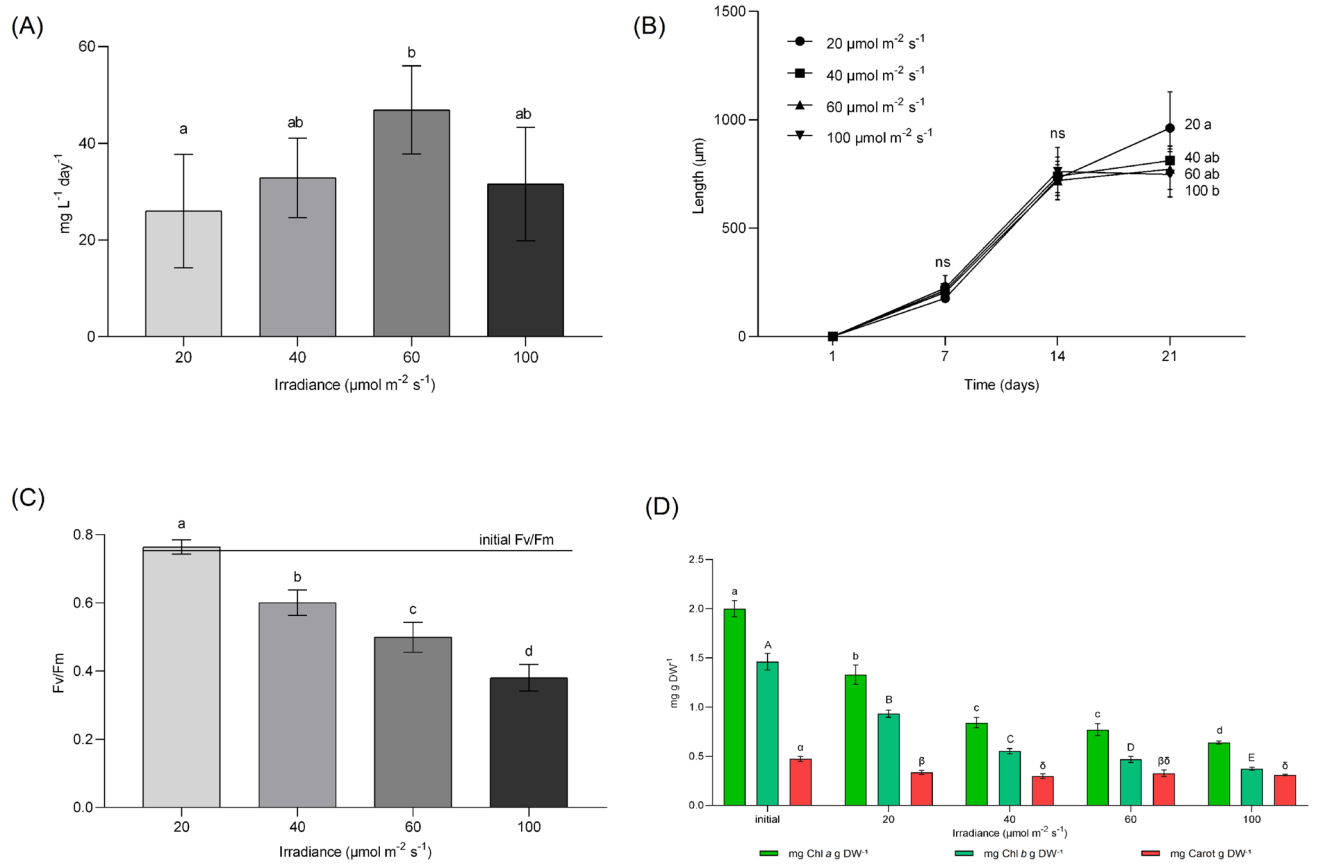


Fig. 2 Effects of light intensity (20, 40, 60 and 100 µmol photons m^{-2} s^{-1}) on (A) productivity (mg DW L^{-1} day^{-1}), (B) filamentous thalli length (µm), (C) maximum quantum yield of photosystem II (F_v/F_m), and (D) pigment content (Chl *a*, Chl *b* and carotenoids; mg g^{-1} DW) of *C. tomentosum*. Filamentous thalli developed from isolated utricles and medullary filaments. Cultures were maintained for 35 days (A, C, D) or 21 days (B) under white light, 16 °C and a 12 h light:12 h dark photoperiod. Initial values (day 1) are shown for F_v/F_m and pigments. Different letters indicate significant differences ($p < 0.05$), and ns indicates non-significant differences. For detailed summary data (mean ± SD) and statistical groupings, see Supplementary material (Table S1)

Pigment concentration was significantly influenced by irradiance for all pigments analyzed (Chl *a*: Welch's ANOVA, $F_{4,15.3} = 553.0$, $p < 0.001$; Chl *b*: $F_{4,45} = 786.3$, $p < 0.001$; carotenoids: Welch's ANOVA, $F_{3,15.7} = 62.5$, $p < 0.001$; Fig. 2D). All pigments showed a significant decrease from initial values across treatments ($p < 0.001$), with a consistent reduction with increasing irradiance. The largest differences were observed between 20 µmol photons m^{-2} s^{-1} (Chl *a*: 1.33 ± 0.10 mg g^{-1} DW; Chl *b*: 0.93 ± 0.04 mg mg g^{-1} DW; carotenoids: 0.34 ± 0.02 mg g^{-1} DW) and 100 µmol photons m^{-2} s^{-1} (Chl *a*: 0.64 ± 0.02 mg g^{-1} DW; Chl *b*: 0.37 ± 0.02 mg g^{-1} DW; carotenoids: 0.31 ± 0.01 mg g^{-1} DW).

Light spectra

Light spectra had a significant effect on the productivity of filamentous thalli of *C. tomentosum* ($F_{3,16} = 5.90$, $p = 0.006$; Fig. 3A). Cultures grown under blue (80.63 ± 20.20 mg DW L^{-1} day^{-1}) and white light (75.76 ± 18.77 mg DW L^{-1} day^{-1}) showed significantly higher productivity than those under red light (33.35 ± 16.41 mg DW L^{-1} day^{-1}; p = 0.0074 and p = 0.0164, respectively), while green light (65.60 ± 22.34 mg DW L^{-1} day^{-1}) did not differ significantly from the remaining treatments.

Filamentous thalli growth was strongly time-dependent ($F_{2,40} = 192.73$, $p < 0.001$), while light spectrum exerted a significant main effect ($F_{3,20} = 7.98$, $p = 0.001$). A significant spectrum × time interaction was also detected ($F_{6,40} = 7.76$, $p < 0.001$; Fig. 3B), indicating that growth trajectories differed among spectral treatments. Across all light spectra, thalli length increased markedly over time ($p < 0.001$), with substantial divergence among treatments emerging at later time points. By day 14, red light produced the highest thalli length (1081 ± 132 µm), and by day 21, white (962 ± 93 µm) and red (896 ± 143 µm) treatments resulted in similar thalli lengths ($p = 0.54$), both remaining significantly higher than blue (717 ± 76 µm) and green light (757 ± 76 µm).

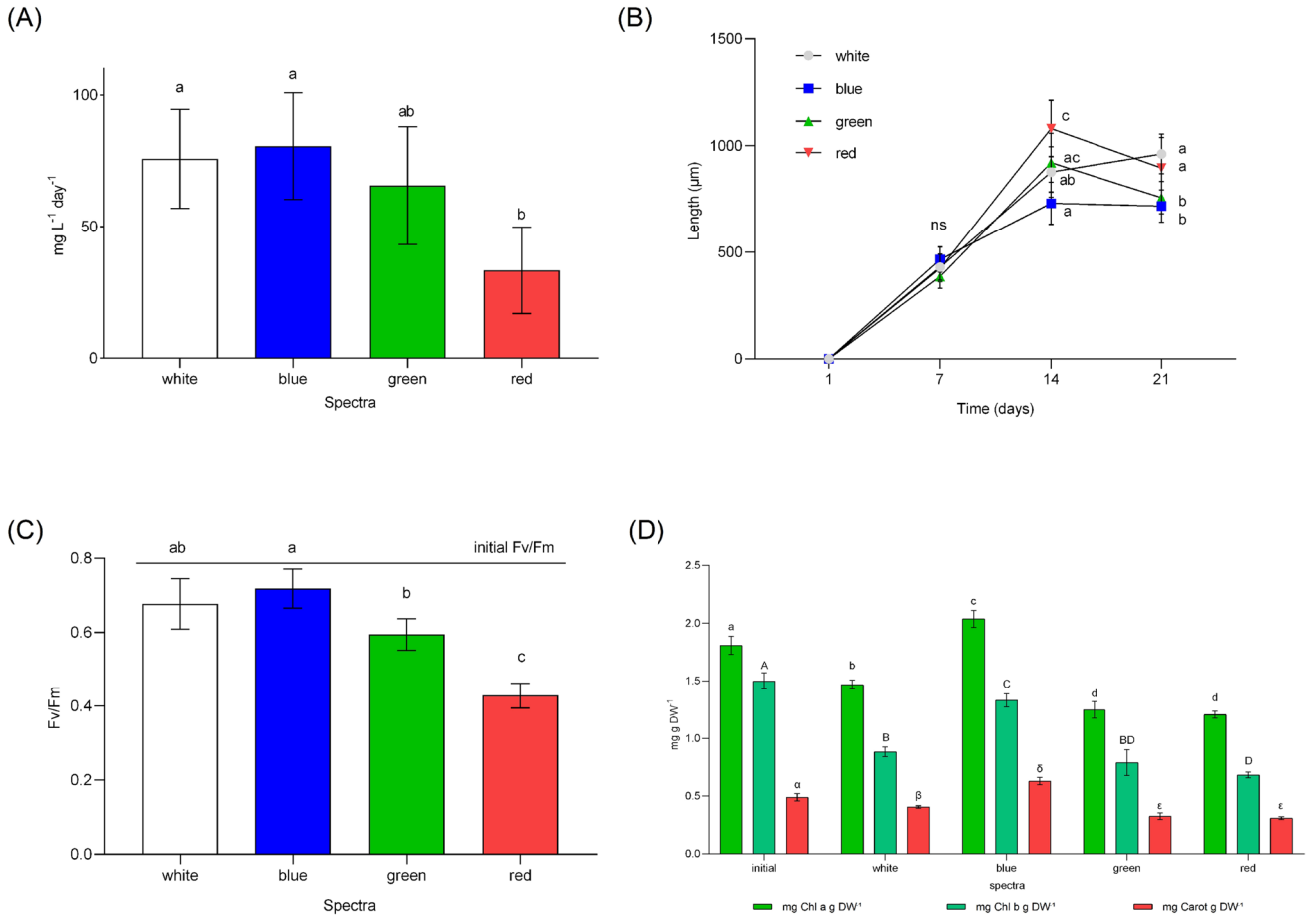


Fig. 3 Effects of light spectra (white, blue, green and red) on (A) productivity (mg DW L^{-1} day^{-1}), (B) filamentous thalli length (µm), (C) maximum quantum yield of photosystem II (F_v/F_m), and (D) pigment content (Chl *a*, Chl *b* and carotenoids; mg g^{-1} DW) of *C. tomentosum*. Filamentous thalli developed from isolated utricles and medullary filaments. Cultures were maintained for 35 days (A, C, D) or 21 days (B) under 40 µmol photons m^{-2} s^{-1}, 16 °C and a 12 h light:12 h dark photoperiod. Initial values (day 1) are shown for F_v/F_m and pigments. Different letters indicate significant differences ($p < 0.05$), and ns indicates non-significant differences. For detailed summary data (mean ± SD) and statistical groupings, see Supplementary material (Table S1)

Photosynthetic performance (F_v/F_m) was strongly influenced by light spectra ($F_{3,20} = 37.75$, $p < 0.001$; Fig. 3C). Cultures under blue light exhibited the highest F_v/F_m (0.72 ± 0.05), significantly higher than green (0.59 ± 0.04; $p = 0.002$) and red light (0.43 ± 0.03; $p < 0.0001$), but not different from white light (0.68 ± 0.07; $p = 0.50$). Red light resulted in the lowest F_v/F_m values among all treatments ($p < 0.001$).

Pigment concentrations were significantly affected by light spectra for all pigments analyzed (chl *a*: Welch's ANOVA, $F_{4,16.9} = 261.0$, $p < 0.001$; chl *b*: Welch's ANOVA, $F_{4,16.5} = 351.0$, $p < 0.001$; carotenoids: Welch's ANOVA, $F_{4,16.6} = 218.0$, $p < 0.001$; Fig. 3D). Blue light promoted the highest chl *a* content (2.04 ± 0.07 mg g^{-1} DW, $p < 0.001$), followed by white light (1.47 ± 0.04 mg g^{-1} DW, $p < 0.001$), whereas green and red light resulted in significantly lower values ($p < 0.001$). Blue light also induced the highest chl *b* concentrations (1.33 ± 0.06 mg g^{-1} DW, $p < 0.001$), significantly exceeding all other light treatments ($p < 0.001$). Carotenoid concentration decreased across treatments except under blue light, which showed the highest value (0.63 ± 0.03 mg g^{-1} DW, $p < 0.001$), whereas the lowest concentrations were observed under green and red light ($p < 0.001$).

Correlations

Correlation analysis revealed strong negative correlation between irradiance and the content of Chl *a* (r = − 0.93), Chl *b* (r = − 0.97), and total Chl (r = − 0.95), indicating a decrease in pigment concentration under higher light intensities (Fig. 4). Conversely, a strong positive correlation was observed between the Chl *a/b* ratio and irradiance (r = 0.78), suggesting a shift in pigment composition in response to light. Carotenoid content showed weak and non-significant

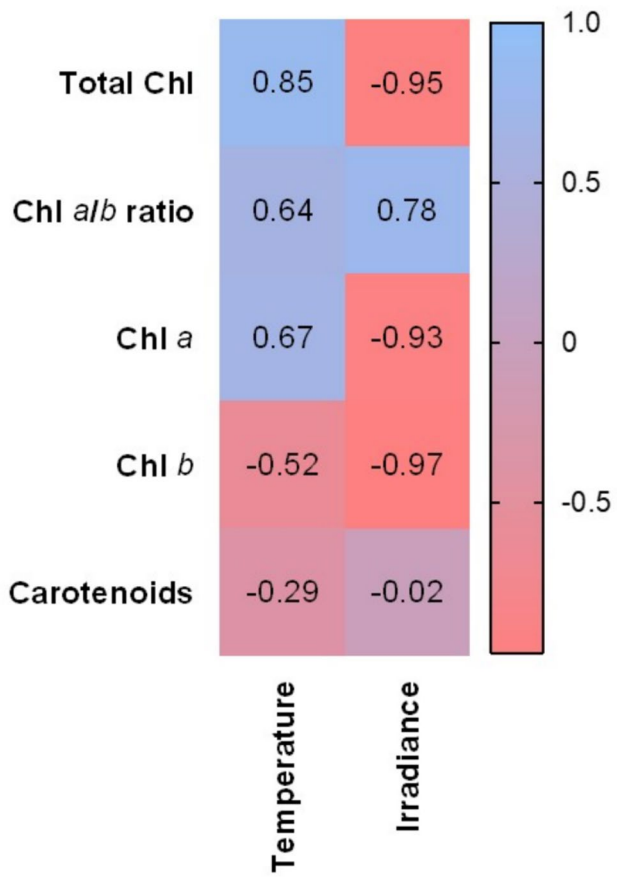


Fig. 4 Correlation analysis between the culture conditions (temperature and light intensity) and the pigment content (Chl *a*, Chl *b*, carotenoids, total Chl (*a*+*b*), and Chl *a*/*b* ratio) of *C. tomentosum* biomass

correlations with both temperature (r = − 0.29) and irradiance (r = − 0.02), indicating little to no influence of these abiotic factors. With respect to temperature, strong positive correlations were detected with total Chl (r = 0.85), and moderate for Chl *a* (r = 0.67) and Chl *a*/*b* ratio (r = 0.64), while Chl *b* showed a moderate negative correlation (r = − 0.52).

Principal component analysis (PCA) revealed clear pigment-based separation among the light spectra treatments (Fig. 5). The first two components explained 99.9% of the total variance (PC1: 88.4%; PC2: 11.5%). Samples under blue light clustered on the negative side of PC1 and were strongly associated with higher Chl *a*, Chl *b*, total Chl, and carotenoid concentrations. In contrast, green and red light treatments grouped on the positive side of PC1, driven mainly by increases in the Chl *a*/*b* ratio. The white light treatment occupied an intermediate position, showing moderate pigment levels. Overall, the PCA shows that blue light enhances total pigment accumulation, whereas green and red light primarily alter pigment proportions, particularly the Chl *a*/*b* ratio.

Discussion

Seaweed growth, development, and physiological performance are governed by the interaction of factors such as temperature, light availability, and light spectra (Endo et al. 2017; Amaro et al. 2024). In the present study, isolated utricles of *C. tomentosum* developed filamentous thalli under all experimental conditions, confirming the high totipotency and regenerative capacity previously described for this genus (Borden and Stein 1969; Park and Sohn 1992; Yang et al. 1997). Similar vegetative development from isolated utricles has been successfully applied in *C. fragile* nursery and seafarm trials, reinforcing the biotechnological potential and relevance of this development stage (Hwang et al. 2005b; 2008).

Productivity of filamentous thalli of *C. tomentosum* remained relatively stable across the tested temperature range (12–20 °C), indicating a broad thermal tolerance during early development. This agrees with earlier reports showing sustained growth of *C. tomentosum* and *C. fragile* filamentous thalli across a wide temperature range, with optimal growth generally occurring near 20–25 °C (Yang et al. 1997). Although temperature had little effect on productivity in our study, filamentous thalli length exhibited temperature-dependent responses, reaching its maximum at 20 °C, after 21 days in culture, suggesting that higher temperatures may accelerate filament elongation. This advantage, however, was accompanied by a decline in maximum quantum yield of photosystem II (F_v/F_m) with increasing temperature. Higher F_v/F_m values were consistently recorded at 12–16 °C, indicating lower physiological stress at cooler conditions. Reduced F_v/F_m values at elevated temperatures are commonly associated with increased non-photochemical quenching (as a heat dissipation mechanism), and in extreme conditions, photoinhibition and thermal damage to PSII reaction centers (Franklin and Forster 1997; Borlongan et al. 2017; Kumar et al. 2020). Temperature exerted a notable influence on the pigment profile of *C. tomentosum*. Chlorophylls *a* and *b* are the primary photosynthetic pigments in green seaweed, playing a crucial role in capturing solar energy and converting it into organic compounds through photosynthesis (Hurd et al. 2014; Sousa et al. 2025). Changes in chlorophyll composition provide insights into the photoacclimation responses of *C. tomentosum* to rising temperatures. The increase in total Chl (*a* + *b*) with temperature may reflect a compensatory investment in light-harvesting capacity, despite declining PSII efficiency. At the same time, the observed moderate positive correlation between the Chl *a*/*b* ratio and temperature suggests a reorganization of the photosynthetic apparatus, a response commonly associated with stress acclimation in macroalgae. A similar response to temperature acclimation was reported by Sousa et al. (2025) for farmed *Ulva lacinulata*, where both total chlorophyll and

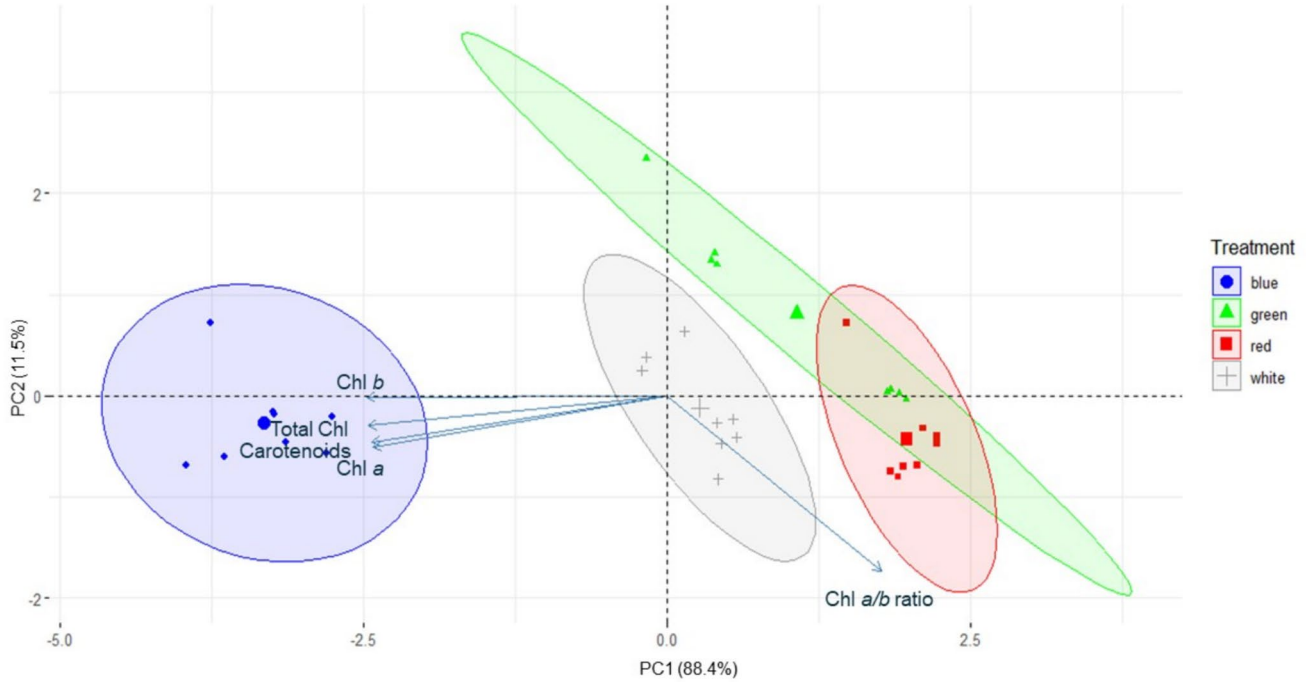


Fig. 5 Principal component analysis illustrating the relationship between the light spectra and the pigment content (Chl *a*, Chl *b*, carotenoids, and total Chl ($a + b$)) and Chl *a*/*b* ratio of *C. tomentosum* biomass

the Chl *a*/*b* ratio positively increased with temperature. In contrast, in the same study, *C. tomentosum* spongy thalli showed only a weak positive correlation between temperature and total chlorophyll content, accompanied by a negative association with the Chl *a*/*b* ratio, suggesting a more limited capacity for antenna reorganization. Carotenoid concentrations remained comparatively stable across temperature treatments. These pigments are generally regarded as more thermally stable than chlorophylls and, beyond their light-harvesting function, contribute to photoprotection through the dissipation of excess excitation energy, the scavenging of reactive oxygen species (ROS), and the modulation of membrane fluidity under thermal stress (Franklin and Forster 1997; Sánchez et al. 2014; Mostafa and Hashem 2025).

Despite the effects of temperature on pigment concentration and elongation of the filamentous thalli, overall productivity remained similar across temperatures, suggesting that other factors, such as light availability, may play a more decisive role in determining growth under the tested conditions. This is consistent with the concept that photosynthetic responses to temperature are strongly dependent on irradiance (Shindo et al. 2022; Terada et al. 2023).

Light absorption is fundamental to initiating the photochemical reactions of photosynthesis, and as such, light availability constitutes the primary factor influencing growth and productivity in seaweed (Hurd et al. 2014). Under low-light conditions, photosynthetic rates increase linearly with irradiance, but this response saturates as light intensity continues to rise, reaching a point where further increases no longer enhance carbon fixation leading to oversaturation and energy loss as heat (Blanken et al. 2016). In the present study, maximum biomass productivity was achieved at an intermediate irradiance (60 µmol photons m^{-2} s^{-1}), while higher irradiance did not further enhance productivity, indicating photosynthetic saturation or early onset of photoinhibition (Blanken et al. 2016; Martins et al. 2023). Comparable patterns have been observed in other *Codium* species, where growth plateaued at moderate light levels despite further increases in photon flux. The filamentous stage of six *Codium* species exhibited growth across a broad range of irradiances (0–175 µmol photons m^{-2} s^{-1}) at 20 °C, but maximum growth occurred at species-specific irradiance levels: 18 µmol photons m^{-2} s^{-1} for *C. bursa* and *C. vermilara*, 18–44 µmol photons m^{-2} s^{-1} for *C. adhaerens*, 44 µmol photons m^{-2} s^{-1} for *C. fragile* ssp. *atlanticum* and *C. tomentosum*, and 88 µmol photons m^{-2} s^{-1} for *C. fragile* ssp. *tomentosoides* (Yang et al. 1997). In the present study, lower irradiance (20 µmol photons m^{-2} s^{-1}) resulted in markedly reduced productivity but promoted increased length of filamentous thalli. This response suggests that low-light conditions may promote filamentous thalli elongation. Similar findings have been reported for *C. fragile*, under low-irradiance conditions (20–40 µmol photons m^{-2} s^{-1}), which promoted filament elongation, whereas higher irradiance (60–100 µmol photons m^{-2} s^{-1}) triggered morphogenesis

into erect spongy thalli (Hwang et al. 2005b). Another filamentous form occurring in sexual reproduction, the germling, also exhibited enhanced length under low-light (20 µmol photons m^{-2} s^{-1}) compared to high-light conditions (40 and 60 µmol photons m^{-2} s^{-1}), both for *C. tomentosum* and *C. fragile* (Hwang et al. 2005b; Sá et al. 2024). These findings emphasize the importance of carefully adjusting irradiance in macroalgal aquaculture systems, so that each life stage, from early development through morphogenesis and later stages, have the most appropriate irradiance, i.e. the minimum necessary energy for maximum electron transport rate (Marques et al. 2022). Maximum quantum yield of photosystem II progressively declined with increasing irradiance, with the highest Fv/Fm values recorded under low-light conditions (20 µmol photons m^{-2} s^{-1}), indicating that isolated utricles and early filamentous stages of *C. tomentosum* are shade-adapted, with PSII functioning most efficiently at low irradiance. This pattern is consistent with previous observations in *Codium* spp., where maximal photochemical efficiency was significantly higher under low light (10–30 µmol photons m^{-2} s^{-1}) and decreased under higher irradiance (90–120 µmol photons m^{-2} s^{-1}; Huang et al. 2025). In the present study, total chlorophyll (*a*+*b*) concentrations were highest under low irradiance and declined with increasing light intensity, while Chl *a*/*b* ratio increased. *C. tomentosum* can modify its photosynthetic apparatus, especially their pigment composition and concentration, to more effectively absorb and utilize the light in their environment. This pattern reflects a coordinated photoacclimation strategy, in which pigment synthesis is upregulated under limiting light to enhance light harvesting and downregulated under high light to reduce excess excitation and oxidative stress (Smith et al. 1990; Marques et al. 2021; Marques et al. 2022). In *C. tomentosum* spongy thalli, exposure to higher irradiance led to a marked reduction in pigments (Chl *a* and Chl *b*), accompanied by an increase in the Chl *a*/*b* ratio, a typical acclimation response to high-light conditions observed in both plants and algae (Giossi et al. 2021). In this study, total carotenoid concentrations remained relatively stable across irradiance treatments. The carotenoid profile of this species is dominated by siphonaxanthin and siphonaxanthin dodecenoate, which function primarily in light harvesting, together with neoxanthin, violaxanthin, and β,ε-carotene, which are associated with photoprotective roles (Giossi et al. 2020; Marques et al. 2021; Takemura et al. 2021; Seki et al. 2022). Under high irradiance, *C. tomentosum* adult spongy thalli have been shown to accumulate trans-neoxanthin and violaxanthin, while reducing siphonaxanthin and siphonaxanthin dodecenoate, indicating a shift from light-harvesting toward photoprotective pigments (Giossi et al. 2021). This internal reorganization of carotenoids likely enhances excess energy dissipation and limits oxidative stress without increasing overall pigment investment. Although the carotenoid profile was not characterized in this study, the stable total carotenoid levels observed are consistent with a dynamic trade-off between light-harvesting and photoprotective pigments, highlighting carotenoid plasticity as a key acclimation mechanism of *C. tomentosum* under variable light conditions.

The spectral quality had pronounced effects on growth, photosynthetic performance, and pigment accumulation in filamentous thalli of *C. tomentosum*. Blue light consistently yielded the highest productivity, maximum quantum yield of photosystem II (F_t/F_m), and pigment concentration (total Chl (*a*+*b*), and carotenoids). White light produced intermediate responses, while red light resulted in the lowest productivity, F_v/F_m values and pigment concentration, but promoted pronounced filament elongation. These contrasting responses suggest that blue light enhances photochemical performance and pigment synthesis, whereas red light favors filamentous elongation. Similar trends have been reported for other seaweed. For example, *Ulva pertusa* and *Ulva lactuca* exhibited higher relative growth rates under blue light compared to red light (Le et al. 2018; Gong et al. 2020), and in *Porphyra umbilicalis*, red light stimulated thallus expansion, cell division, and polysaccharide accumulation, whereas blue light primarily enhanced pigment and protein synthesis (Figueroa et al. 1995). Noteworthy, Marques et al. (2021) reported that adult thalli of *C. tomentosum* exhibited higher growth rates under red light compared to white or blue light. These contrasting findings reinforce that the physiological effects of spectral light quality are not only species-specific but may be also dependent on developmental stage (Amaro et al. 2024). Red light may be advantageous for promoting filamentous thalli elongation in *C. tomentosum,* preserving biomass from a biobanking perspective, whereas blue and white light appear more suitable for promoting higher photosynthetic efficiency and vegetative growth during nursery stages. In this study, pigment content was modulated in response to light spectrum, reflecting a chromatic acclimation to optimize light absorption under variable light conditions, as observed for other seaweed (Le et al. 2018). Blue light significantly stimulated the synthesis of Chl *a*, Chl *b*, and carotenoids, whereas red light resulted in consistently lower pigment accumulation. These results are consistent with Marques et al. (2021), who reported higher concentrations of Chl *a*, Chl *b*, and carotenoids in adult spongy thalli of *C. tomentosum* cultured under blue light compared to white and red light. Green light consistently induced intermediate pigment responses, which can be explained by the presence of siphonaxanthin-based light-harvesting complexes characteristic of siphonous green algae. These complexes enable efficient absorption of blue–green wavelengths, which dominate the light environment where *C. tomentosum* naturally occurs (Hurd et al. 2014; Giossi et al. 2021). In contrast, white light—comprising a full spectrum of wavelengths

and simulating natural sunlight—resulted in intermediate to high pigment concentrations, likely due to the simultaneous activation of multiple pigment systems (Hurd et al. 2014; Mascoli et al. 2020). Together, these results underscore the physiological advantages of blue and white light over red light in supporting pigment accumulation and photosynthetic performance in *C. tomentosum* filamentous stage.

Overall, our results highlight that *C. tomentosum* early developmental stages are more sensitive to light intensity and spectral quality than to temperature within the tested range. Although this species exhibits a broad thermal tolerance, higher temperature promoted elongation of filamentous thalli, whereas lower temperatures enhanced photosynthetic efficiency. Moderate irradiance maximized overall productivity, low light favored filament elongation, and blue and white lights enhanced photosynthetic efficiency and pigment accumulation. In contrast, red light constrained photosynthetic efficiency but may be advantageous for biobanking strategies by maintaining elongated filamentous biomass under reduced metabolic activity. Fine-tuning irradiance and spectral composition according to developmental stage can therefore improve biobank and nursery efficiency, reduce production time, and support scalable cultivation of *C. tomentosum* for aquaculture and biotechnological applications.

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Data availability The datasets generated and/or analyzed in this study are available from the corresponding author upon reasonable request.

Declarations

Conflicts of interest The authors declare no competing interests.

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