Effects of photoperiod and light spectra on growth and pigment composition of the green macroalga *Codium tomentosum*

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Abstract

Codium tomentosum is a marine green macroalga with multiple value-added applications that is being successfully used as an extractive species in sustainable integrated multi-trophic aquaculture systems. Nonetheless, growth conditions of this species at an early development phase still require optimization. The present study addresses, under controlled laboratory conditions, the effects of photoperiod (long vs. short-day) and light spectra (white, blue, and red light) on growth and pigment composition of *C. tomentosum*. Relative growth rate was approximately 2× higher under long-day photoperiod (average of 39.2 and 20.1% week^{-1} for long and short-day, respectively). Concentrations per dry weight of major pigments such as chlorophyll *a* (Chl*a*) and siphonoxanthin (Siph) were significantly higher under long-day photoperiod. Relative growth rates were higher under red light, intermediate under white light, and lower under blue light. These last results were rather surprising, as Siph-Chl*a*/Chl*b* light harvesting complexes of *Codium* have increased absorption in the blue-green region of the light spectra. Changes in carbon allocation patterns caused by the spectral composition of light and overgrowth of green microalgae in blue light cultures could explain the differences recorded for relative growth rate. Long-day photoperiod and light sources with preferential emission at the red region of the light spectra were identified as optimal for growth of *C. tomentosum* at early development stages. These lighting conditions can reduce the time required to reach the necessary biomass before transfer to grow-out systems. Overall, these findings can shorten production time, increase macroalgal productivity, and enhance aquaculture revenues.

Keywords Chlorophyta · Aquaculture · Growth rate · Irradiance · Nursery · Photosynthesis · Productivity

Introduction

Environmental concerns related to traditional fish aquaculture practices include oxygen depletion, eutrophication, and algal blooms, resulting from nutrient-enriched effluent discharges to adjacent aquatic environments (Pillay 2004). Integrated multi-trophic aquaculture (IMTA) approaches have emerged, as these include extractive species from different trophic levels to uptake available nutrients in effluent waters. This practice is perceived as more environmentally friendly and economically sustainable (Granada et al. 2016). The inclusion of certain macroalgal species in IMTA systems allows the reduction of costs of lowering inorganic nutrient loads in aquaculture effluents and the production of an additional value-added product (Troell et al. 2003). Macroalgae are important natural resources explored for a variety of purposes, mainly as food and feed, but also as biofuels, biostimulants, and cosmetics (Samaraweera et al. 2011; Abreu et al. 2014; Santos et al. 2015).

Codium is a genus of siphonous green macroalgae (Chlorophyta) that is either farmed or foraged from the wild. *Codium* species are known to be an important source of sulfated polysaccharides (Wang et al. 2014) and other compounds with multiple bioactive properties, such as antioxidant, antiviral, anticoagulant, antiangiogenic, and antitumoral

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activities (Matsubara et al. 2000; Ohta et al. 2009; Ganesan et al. 2010, 2011; Rey et al. 2020). Recently, farmed biomass of the native Atlantic species *Codium tomentosum* was shown to be a valuable source of important phytochemicals, such as stigmasterol and α-tocopherol (Santos et al. 2015), also being rich in polyunsaturated fatty acids with nutritional and health benefits (Costa et al. 2015). Extracts of *C. tomentosum* are already being successfully used as a moisturizing agent in the cosmeceutical industry (Wang et al. 2015; Pereira 2018). *Codium* is also ranked amongst one of the most popular edible macroalgae and is listed as a delicacy in gourmet cuisine (Radulovich et al. 2015; Pérez-Lloréns et al. 2018).

The pigment composition of siphonous green macroalgae is characterized by the presence of the keto-carotenoid siphonaxanthin and its ester siphonaxanthin dodecenoate (siphonein) as the main light-harvesting carotenoids (Anderson 1983; Wang et al. 2013). Light-harvesting complexes of siphonous green alga are considered an evolutionary relic of some Chlorophyta, in which the presence of siphonoxanthin and chlorophyll *b* allows an increased absorption in the green and blue-green light region (Anderson 1983; Akimoto et al. 2007; Wang et al. 2013). Another peculiarity of siphonous green macroalgae of order Bryopsidales, which includes *Codium* spp., is the absence of zeaxanthin as a nonphotochemical quencher (Cruz et al. 2015; Christa et al. 2017). Whereas higher plants and most algae rely on a xanthophyll cycle to dissipate excessive absorbed light energy as heat under high irradiance, Bryopsidales macroalgae lack this important photoprotection mechanism (Christa et al. 2017). It is still not clear which are the main mechanisms contributing to the protection of Bryopsidales from excessive light exposure.

Studies addressing optimal growth conditions for *Codium* species are still scarce. Yang et al. (1997) observed that filamentous thalli of several *Codium* species grew over a broad range of environmental conditions. Higher growth rates of *C. fragile* ssp. *tomentosoides* were obtained with temperatures ranging from 18 to 24 °C and a long-day photoperiod of 16 h of light per day (Hanisak 1979). Hwang et al. (2008) observed that optimal light doses for growth of *C. fragile* differed according to culture stage. Variation of the optimum light requirements for the latter species suggests that the different life stages may be sensitive not only to total irradiance but also to spectral composition (Hwang et al. 2008).

The species *C. tomentosum* is currently produced in a land-based organic certified IMTA system operated by the company ALGAplus (Portugal) using nutrient-rich effluent from earthen ponds employed for the semi-intensive farming of European sea bass and gilthead sea bream. The company has initiated the cultivation of *C. tomentosum* in 2013, having developed proprietary protocols for year-round production of this species, combining an initial hatchery phase under controlled temperature, photoperiod, and light conditions with an outdoor phase where *C. tomentosum* is grown in free-floating conditions. As the company operates several macroalgae species, the conditions in the hatchery phase are not optimized for *C. tomentosum*. Thus, in this study, we tested under controlled laboratory conditions the effects of photoperiod (long vs. short-day; LD and SD, respectively) and light spectra (white, blue, and red light; WL, BL, and RL, respectively) on the growth and pigment composition of *C. tomentosum* at an initial growth phase. Optimizing growth conditions at this stage can reduce the time required at an initial development phase, allowing a faster transfer to grow-out systems and reducing farming operational costs. Ultimately, by fine-tuning lighting practices at this early step of production, it may be possible to more rapidly satisfy the current growing demand by the market of *Codium* biomass.

Materials and methods

Biological material and culturing conditions

Domesticated thalli of *Codium tomentosum* (Stackhouse, 1797) were supplied in February 2019 by the company ALGAplus (variety C2.21.AC.160309). For acclimation purposes, the macroalga was grown for 2 weeks, under a light intensity of 230 µmol photons m^{-2} s^{-1}, a temperature of 17 ± 1 °C, and two photoperiods: long-day (16 h light:8 h dark) and short-day (8 h light:16 h dark). Illumination was provided by OSRAM Fluora T8 58W/77 fluorescent lamps. Each culture was grown in 1 L of culture medium in 2-L flasks with aeration strong enough to move the thalli around. Irradiance was quantified inside the culture flasks using a US-SQS/L spherical micro quantum sensor connected to a Universal Light Meter ULM-500 (Walz, Germany). Culture media was the same used at the experimental trials conducted by ALGAplus, consisting of autoclaved nutrient-rich seawater supplemented with VSE and germanium dioxide to prevent the growth of unwanted diatoms in experimental cultures.

Photoperiod experiment

The photoperiod experiment was designed to test the effects of two different photoperiods in the growth of *C. tomentosum*: long-day (16 h light:8 h dark; LD) and short-day (8 h light:16 h dark; SD). After the acclimation period described above, the macroalga was monitored over 5 consecutive weeks with 5 replicates per photoperiod. Algal culture maintenance was performed weekly, with glassware and medium being replaced and algal biomass stocking density adjusted to initial density (10–15 apices of *C. tomentosum* of ca. 2 cm of length per liter of culture).

Light spectra experiment

The light spectra experiment was designed to test the effects of three different light spectra in the growth of *C. tomentosum*: white, red, and blue light (WL, RL, and BL, respectively). The same quantum irradiance of 120 µmol photons m^{-2} s^{-1} was used for WL (consisting in the total spectrum of OSRAM Fluora T8 fluorescent lamps), RL (a red Lee Filter allowing the red part of the light spectrum to be transmitted), and BL (a blue Lee Filter allowing the blue part of the light spectrum to be transmitted). Light spectra (Fig. 1) were measured using an OceanOptics spectrometer (Flame model, wavelength range 190–1100 nm). The macroalga was monitored for 5 consecutive weeks at long-day photoperiod (16 h light:8 h dark) and at 17 ± 1 °C. This photoperiod was used due to the higher growth rates observed (see "Results"). Maintenance of algal cultures was performed weekly as described above, with algal biomass density adjusted to the initial density.

Biomass quantification

Macroalgal biomass was quantified every week. The macroalga was transferred to a Petri dish and excess of growth medium removed with two pieces of paper towel. The fresh biomass was then weighted using a precision scale. Relative growth rate (RGR) was calculated according to the equation $[Ln(W_f) - Ln(W_i)]/t \times 100$, where W_f is the final fresh weight and W_i is the initial fresh weight, while t refers to time expressed in weeks.

Chlorophyll fluorescence

In vivo chlorophyll (Chl) *a* fluorescence was measured using an Imaging-PAM fluorometer Mini version (Walz, Germany).
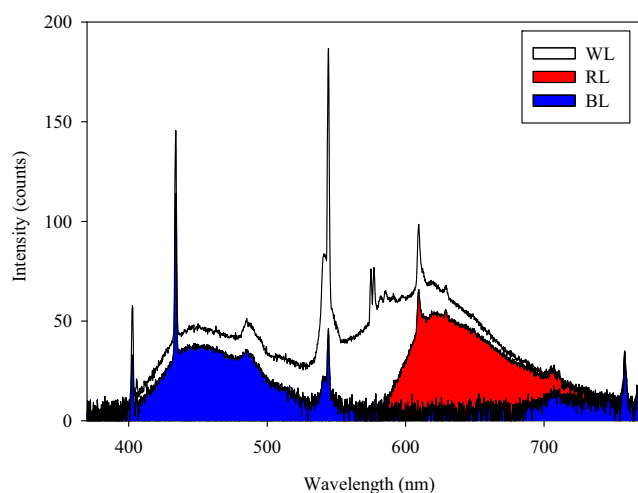

Fig. 1 Emission spectra of growth lights (WL—white light, OSRAM Fluora T8 fluorescent lamps; RL—red light; and BL—blue light). RL and BL were obtained using wavelength-selective filters over white light

A characterization of photosynthetic activity was performed through steady-state light-response curves (SSLC) measured at the end of each experimental period (week 5). First, the macroalgal samples were dark-acclimated for 15 min and maximum quantum yield of photosystem (PS) II (F_v/F_m) was determined by calculating $(F_m - F_o)/F_m$, where F_m and F_o are the maximum and the minimum fluorescence of the dark-adapted samples, respectively (Murchie and Lawson [2013]). Secondly, measurements of the effective PSII quantum yield were obtained at the end of 9 steps of 5 min of increasing irradiances up to 604 µmol photons m^{-2} s^{-1}. Measurements were done at constant temperature (17 ± 1 °C) and at the same time of the day (measurements started 6 h after the onset of light). Steady-state light-response curves were constructed by calculating, for each level of actinic light, the relative electron transport rate (rETR) at the delivered actinic photon irradiance (*E*), and the effective quantum yield of PSII ($\Delta F/F_m'$) as rETR = $E \times (F_m' - F)/F_m'$, where F_m' and F are the maximum and the steady-state fluorescence of light exposed samples, respectively (Genty et al. [1989]). Numerical values of variable Chl fluorescence parameters were extracted from the digital images using the imaging system software (Imaging Win, Heinz Walz GmbH, Germany) by selecting areas of interest. Light curves were characterized by fitting the model of Platt et al. ([1980]). The estimated parameters were (i) α (initial slope of ETR versus E curve), representing the light utilization coefficient and related to the photosynthetic efficiency at limiting irradiances; (ii) rETR$_{max}$ (maximum relative ETR) related to the photosynthetic capacity at saturating irradiances. The model was fitted iteratively using MS Excel Solver. The curve fits obtained were always considered as very good ($r > 0.98$).

Photosynthetic pigments analysis

At the end of the experimental periods, macroalgal material was flash-frozen in liquid nitrogen and freeze-dried prior to photosynthetic pigment analysis. Pigment quantification by high-performance liquid chromatography (HPLC) followed the C18 method described by Mendes et al. ([2007]). For every extraction, the amount of lyophilized sample used followed the ratio of 40 mg per 1 mL of extraction solution (95% cold buffered methanol with 2% ammonium acetate). Samples were ground with a plastic rod and sonicated for 1 min. Samples were then transferred to − 20 °C for 20 min in the dark. Extracts were filtered through 0.2 µm PTFE membrane filters and immediately injected into a Prominence-*i* LC 2030C HPLC system (Shimadzu, Japan) with a photodiode array detector. Chromatographic separation was carried out using a Supelcosil C18 column (250 mm length; 4.6 mm diameter; 5 µm particles; Sigma-Aldrich, USA) for reverse-phase chromatography and a 35 min elution program. The solvent gradient followed Kraay et al. ([1992]), with an injection volume of 50 µL and a flow rate of 0.6 mL min^{-1}.

Pigments were identified from absorbance spectra and retention times and concentrations calculated from the signals in the photodiode array detector in comparison with pure crystalline standards from DHI (Hørsolm, Denmark). The concentration of each pigment was expressed per dry weight (μmol g dw^{-1}).

Statistical analysis

Significant differences in relative growth rate were tested using a mixed ANOVA (within-subjects factor, time; between-subjects factor, light treatment), after checking for homogeneity of variances (Levene's Test), normality (Shapiro-Wilk's test), and sphericity (Mauchly's test). Multiple comparisons were performed with Tukey's test with significance levels adjusted by the Bonferroni correction. For the fluorescence parameters (F_v/F_m, α, rETR$_{max}$, and E_k) and pigment concentrations, differences between photoperiods (long- and short-day) were tested for significance using a t test, while those between light spectral treatments (white, red, and blue light) were investigated using one-way ANOVA. The nonparametric Kruskal-Wallis test was used when data violated assumptions of normality or homogeneity of variances. Statistical analysis was done using SPSS Statistics v25.0 for Windows.

Results

Photoperiod experiment

The relative growth rate (RGR) of *C. tomentosum* under long-day (16 h light:8 h dark; LD) photoperiod was significantly higher ($F_{1,8} = 129.8$, $p < 0.001$) than that recorded under short-day (8 h light:16 h dark; SD) conditions (Fig. 2). Multiple comparisons revealed significant differences between light treatments (in all cases $p < 0.001$) for all time points (weeks). Average RGR observed were 39.2 ± 3.2 and $20.1 \pm 1.9\%$ week^{-1} for LD and SD, respectively. Within each treatment, along the 5-week growth period, *C. tomentosum* cultures growth rates decreased from week 1 to week 3, but the RGR values leveled on week 4 and 5.

Maximum quantum yield (F_v/F_m) of *C. tomentosum* at week 5 was 0.73 ± 0.01 under both LD and SD photoperiods. Light curves were also similar between photoperiods, with relative electron transport rate increasing with irradiance and saturating at light intensities ca. 200 μmol photons m^{-2} s^{-1} (Fig. 3). No significant differences were observed between LD and SD treatments for steady-state light-response curves (SSLC) parameters α (0.90 ± 0.11 and 0.80 ± 0.09, respectively) and rETR$_{max}$ (26.8 ± 4.0
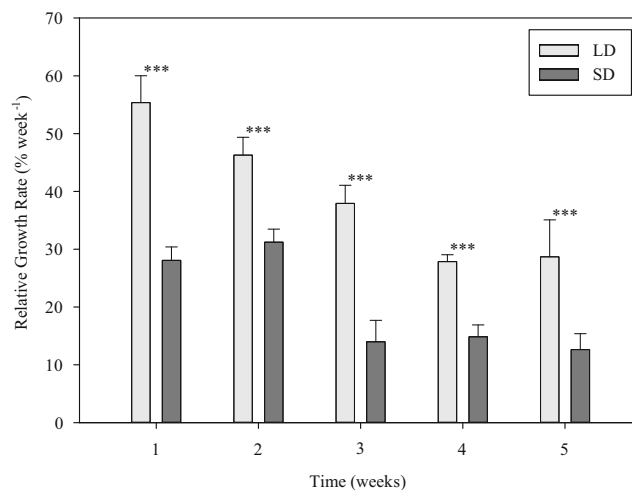

Fig. 2 Relative growth rate of *Codium tomentosum* (mean ± SD, $n = 5$) along a 5-week growth period under long-day photoperiod (16 h light:8 h dark, LD) and short-day photoperiod (8 h light:16 h dark, SD). Differences between light treatments were significant in all cases at $p < 0.001$ (triple asterisk)

and 27.0 ± 1.8, respectively). However, Chl fluorescence parameters revealed high spatial heterogeneity within the macroalgal thallus. A gradient was observed with higher F_o values and lower photosynthetic yields registered at the macroalgal tips, when compared with basal areas of the thallus (Fig. 4).

Pigments identified in *C. tomentosum* by HPLC included (by elution order) siphonoxanthin (Siph), all-*trans*-neoxanthin (*t*-Neo), 9′-*cis*-neoxanthin (*c*-Neo), violaxanthin (Viola), siphonaxanthin dodecenoate (Siph-do), chlorophyll *b* (Chl*b*), chlorophyll *a* (Chl*a*), and β,ε-carotene (βε-Car) (Table 1). Average pigment concentrations were higher under LD
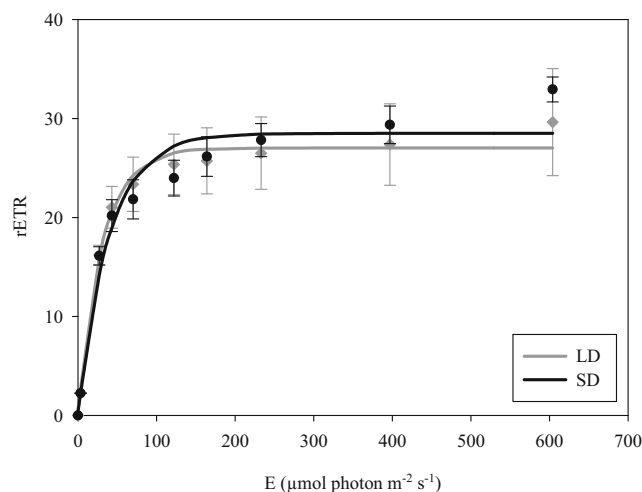

Fig. 3 Steady state light-response curves of relative electron transport rate (rETR) vs. photon irradiance (E) in *Codium tomentosum* (mean ± SD, $n = 5$) after 5 weeks under long-day photoperiod (16 h light:8 h dark, LD) and short-day photoperiod (8 h light:16 h dark, SD). Lines represent curve fits according to the model by Platt et al. (1980)

Fig. 4 Fluorescence heterogeneity in *Codium tomentosum*. **a** Maximum fluorescence (F_m) map measured in 15 min dark-adapted samples of *C. tomentosum*. **b** Minimal fluorescence (F_o) and maximum photosynthetic yield (F_v/F_m) in transect 0–321 represented in **a**
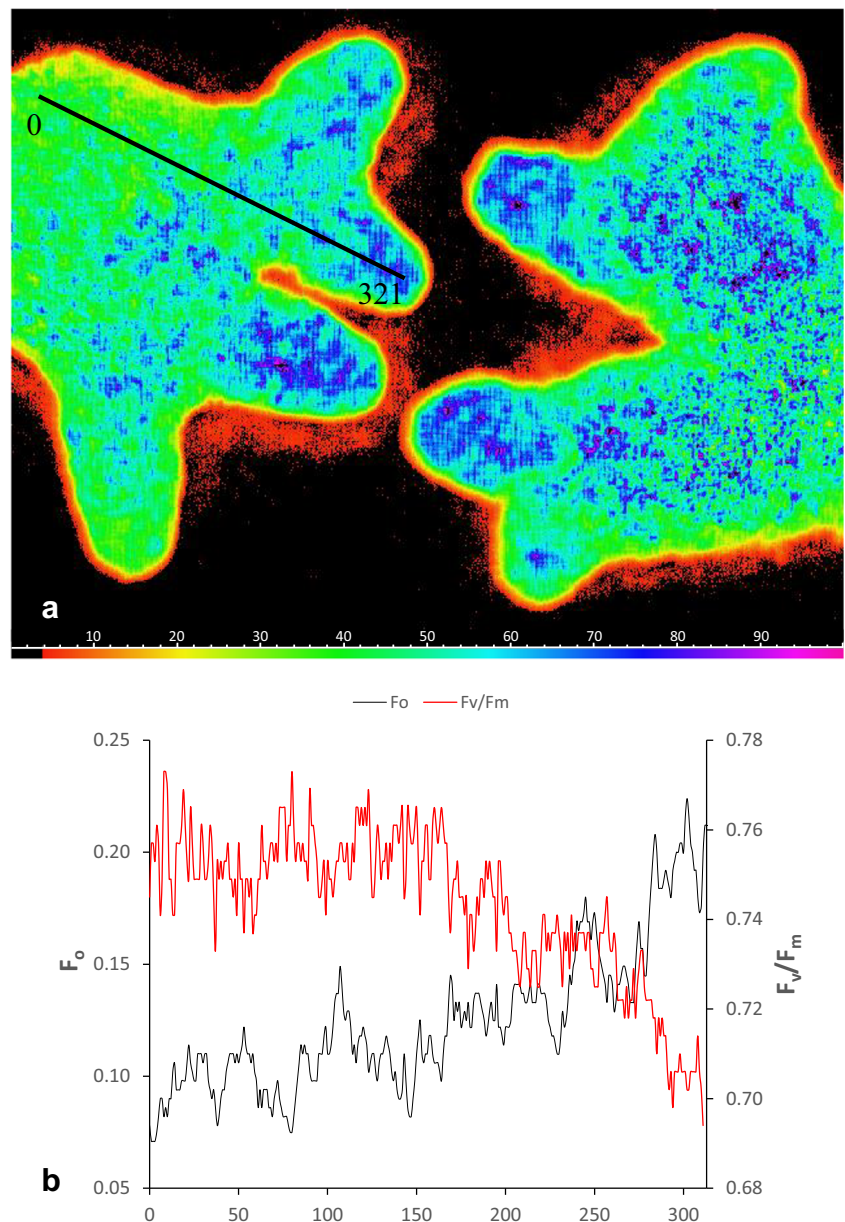
conditions for all the pigments identified (Fig. 5). However, pigment concentrations were only significantly higher ($p < 0.05$) under LD conditions for Siph, *c*-Neo, Viola, and Chl*a*. The remaining pigments (*t*-Neo, Siph-do, Chl*b*, and

Table 1 List of photosynthetic pigments identified in *Codium tomentosum*, with average retention times and absorption maxima (λ_{max})

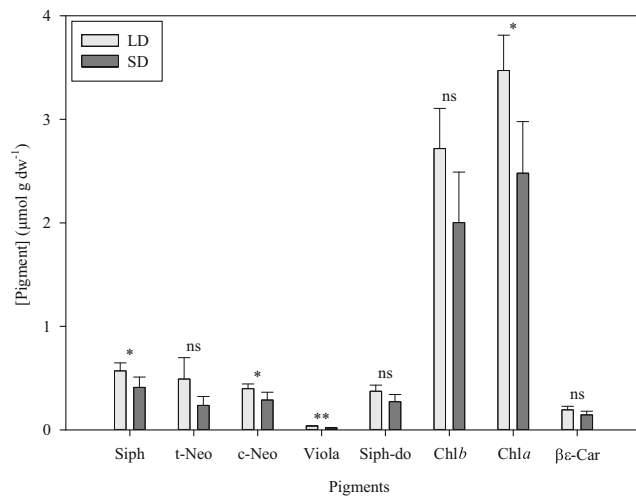| Pigments	Retention time (min)	λ_{max} (nm)
Siphonoxanthin (Siph)	10.51	448
All-*trans*-neoxanthin (*t*-Neo)	11.49	418, 441, 471
9′-*cis*-neoxanthin (*c*-Neo)	12.12	413, 437, 466
Violaxanthin (Viola)	13.11	417, 441, 471
Siphonaxanthin dodecenoate (Siph-do)	18.67	455
Chlorophyll *b* (Chb)	22.85	458, 595, 645
Chlorophyll *b* (Cha)	24.40	430, 622, 667
β,ε-carotene (βε-Car)	28.53	447, 475

Fig. 5 Pigment content in *Codium tomentosum* (mean ± SD, $n = 4$) at week 5 under long-day photoperiod (16 h light:8 h dark, LD) and short-day photoperiod (8 h light:16 h dark, SD). Differences between light treatments were nonsignificant (ns), significant at $p < 0.05$ (single asterisk), or significant at $p < 0.01$ (double asterisk)

$\beta\epsilon$-car) showed no significant difference between photoperiods.

Light spectra experiment

There was a significant effect of light spectral treatment ($F_{2,12} = 33.8$, $p < 0.001$) on the RGR of *C. tomentosum* (Fig. 6). RGR was significantly higher under RL, intermediate in WL, and lower in BL (in all cases $p < 0.05$). Average RGR observed were 36.7 ± 2.7, 31.5 ± 1.4, and $21.8 \pm 3.3\%$ week^{-1} for RL, WL, and BL, respectively. Along the 5-week growth period, *C. tomentosum* growth rates were consistently lower

under BL, whereas differences between RL and WL were only statistically significant ($p < 0.01$) for weeks 1 and 2.

Maximum quantum yield (F_v/F_m) values of *C. tomentosum* at week 5 under WL, BL, and RL treatments were similar (0.69 ± 0.03, 0.68 ± 0.04, and 0.71 ± 0.02, respectively). Light curves were also similar for light spectral treatments, with rETR increasing with irradiance and saturating at light intensities slightly higher than the growth irradiance of 120 μmol photons m^{-2} s^{-1} (Fig. 7). Relative electron transport rate slightly decreased under higher irradiances (397 and 604 μmol photons m^{-2} s^{-1}) for WL grown alga, indicating photoinhibition. However, no significant differences were observed between WL, BL, and RL treatments for SSLC parameters α (0.69 ± 0.05, 0.78 ± 0.09 and 0.75 ± 0.05, respectively) and rETR$_{max}$ (33.3 ± 3.6, 38.2 ± 3.7 and 35.9 ± 4.3, respectively).

No significant differences were observed between light spectral treatments in the concentrations of the pigments *t*-Neo, Viola, and Chl*a*. However, the average concentrations for the majority of identified pigments were higher under BL (Fig. 8). Concentrations of *c*-Neo, Siph-do, and Chl*b* were significantly higher ($p < 0.05$) under BL than RL and WL. Concentrations of Siph and $\beta\epsilon$-car were significantly higher under BL than RL.

Discussion

Seaweed growth, reproduction, and chemical composition are affected by the interaction of temperature, nutrients, and light (Lüning 1990; Lobban and Harrison 1994). Thus, understanding the combined effects of these parameters is crucial for any long-term commercial algae farm operation (Pereira et al.
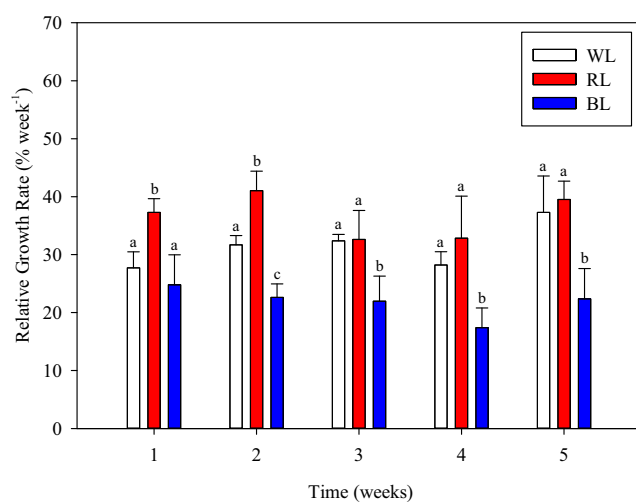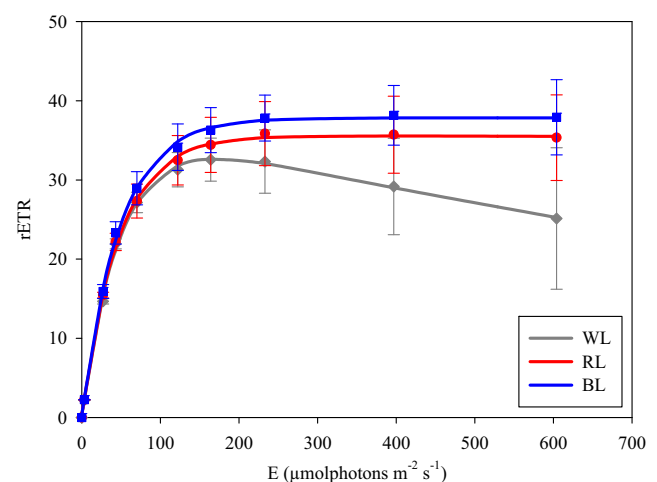

Fig. 6 Relative growth rate of *Codium tomentosum* (mean ± SD, $n = 5$) along a 5-week growth period under white light (WL), red light (RL), and blue light (BL). Different letters indicate, for each week, significant differences between light treatments at $p < 0.05$

Fig. 7 Steady state light-response curves of relative electron transport rate (rETR) vs. photon irradiance (E) in *Codium tomentosum* (mean ± SD, $n = 5$) after 5-weeks under white light (WL), red light (RL), and blue light (BL). Lines represent curve fits according to the model of Platt et al. (1980)
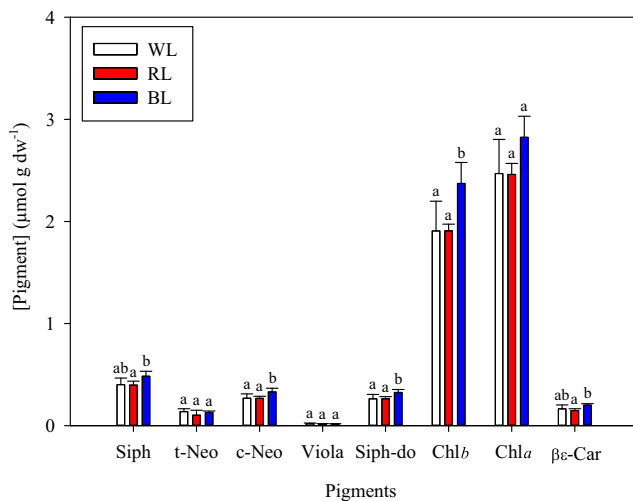
Fig. 8 Pigment content in *Codium tomentosum* (mean ± SD, *n* = 5) at week 5 under white light (WL), red light (RL), and blue light (BL). Different letters indicate significant differences between light treatments at *p* < 0.05

2004; Abreu et al. 2011). Relative growth rates of the macroalga *C. tomentosum* under culture conditions were ca. 2× higher under long-day photoperiod (LD, 16 h light:8 h dark) compared with short-day photoperiod (SD, 8 h light:16 h dark). Main photosynthetic parameters determined by PAM fluorometry were identical between LD and SD, indicating that higher productivity under LD conditions were likely due to the longer period of light exposure and not to changes in photosynthetic efficiency.

According to Hanisak (1979), seasonal growth of *C. fragile* ssp. *tomentosoides* is primarily determined by the interaction of temperature and irradiance. In culture experiments, higher growth rates of this *Codium* species were observed at 18 and 24 °C under long-day photoperiods (Hanisak 1979). However, a decrease in growth rate was observed for the combination of higher temperature and irradiance (24 °C, 150 μmol photons m^{-2} s^{-1}). *Codium fragile* culture experiments were conducted by Hwang et al. (2008) using seed fibers and cultivation ropes. Higher growth rates for pre-main cultivation stage of *C. fragile* were obtained at 2 m depth, corresponding to a mean irradiance of ca. 250 μmol photons m^{-2} s^{-1} (Hwang et al. 2008). Nanba et al. (2005) observed that the growth of the spongy thalli of *C. fragile* greatly accelerated under the combination of higher irradiance and water flow (200 μmol photons m^{-2} s^{-1} and 10 cm s^{-1}, respectively). In our study, despite ca. 2× fold higher irradiance in the photoperiod experiment (230 μmol photons m^{-2} s^{-1}), compared with the light spectra experiment (120 μmol m^{-2} s^{-1}), average relative growth rate under WL was only 20% higher (39.2 and 31.5% week^{-1}, respectively). Hence, we conclude that optimum light intensity for *C. tomentosum* growth is within the 120–230 μmol photons m^{-2} s^{-1} range.

In most cases, studies on the effect of light on the growth of macroalgae involve manipulation of the integrated photosynthetically active photon irradiance, while spectral composition is commonly overlooked. However, spectral composition of available light may significantly affect macroalgal growth (Figueroa et al. 1995). Relative growth rate observed for *C. tomentosum* was significantly higher under red light than under blue light (36.7 and 21.8% week^{-1}, respectively). This was rather surprising considering that Sipho-type light-harvesting complexes of *Codium* have a higher absorption in the blue-green region of the light spectra (Anderson 1983; Benson and Cobb 1983). Main photosynthetic parameters determined by PAM fluorometry were identical between blue and red light, showing that spectral light acclimation did not affect the photochemical reactions of photosynthesis. However, spectral light acclimation may have significantly affected the metabolic use of energy and carbon allocation patterns. Figueroa et al. (1995) cultivated the macroalga *Porphyra umbilicalis* for 4 weeks in either blue or red light at the same photon flux density and found higher growth rates under red light. The authors observed that red light favored thallus expansion, cell division, and polysaccharide production, whereas blue light favored pigment and protein synthesis. Jungandreas et al. (2014) studying light acclimation processes in the microalgae *Phaeodactylum tricornutum* found that red to blue light shift led to a redirection of carbon into the synthesis of proteins, whereas during the blue to red light shift an accumulation of carbohydrates occurred. These differences in carbon partitioning significantly affected growth with severe inhibition in the red to blue transition (Jungandreas et al. 2014). Noteworthy was the overgrowth of green microalgae observed in blue light cultures of *C. tomentosum*. Competition of microalgae with *C. tomentosum* for available light and nutrients could also explain the reduced productivity recorded in the present study under blue light.

Photosynthetic organisms, such as macroalgae, are able to manipulate the composition of their photosynthetic apparatus, in particular their pigment composition, in order to optimize their light harvesting properties. Photoperiod affected pigment composition of *C. tomentosum*, with significantly higher concentrations of major light harvesting pigments such as Chl*a* and Siph under long-day conditions. Spectral composition of light also affected pigment composition of *C. tomentosum*, although concentrations were similar under white and red light. On the contrary, concentrations were generally higher under blue light, particularly for Siph, *c*-Neo, Siph-do, Chl*b*, and βε-car. Blue light has been shown to cause higher chlorophyll production in microalgae (Jeffrey 1980; Senge and Senger 1991). In the green microalgae *Chlorella* and *Ankistrodesmus*, blue light caused an increase in total chlorophylls that was more pronounced for Chl*b* (Senge and Senger 1991).

Siphonous green algae differ from other Chlorophyta in containing light harvesting complexes with xanthophylls Siph and Siph-do, as well as a higher proportion of Chl*b* (Anderson 1983; Wang et al. 2013). In *C. tomentosum*, Siph and Siph-do pigments accounted for 54% of the total carotenoid content. Benson & Cobb (1981) observed that Siph and Sipho-do accounted for 60% of total carotenoids in *C. fragile*. In *C. tomentosum*, Chl *a*/*b* ratios were low (~ 1.3) reflecting the large proportion of Chl*b* in comparison with the light-harvesting complexes of plants (Anderson et al. 1988). The high proportion of Siph, Siph-do, and Chl*b* has been related to enhanced light harvesting in deep or turbid coastal waters with higher proportions of available green and blue-green light (Anderson 1983; Benson and Cobb 1983; Wang et al. 2013). In turbid coastal waters, high absorption and scattering of longer wavelengths by dissolved or suspended particles make the ability to exploit the spectral region of blue-green light an ecological advantage (Kirk 2011). Our results suggest, however, that the evolutionary pressures on *C. tomentosum* that led to an increased ability to explore the blue-green region of the spectrum did not lead to a downregulation of the capability to use the spectral red region.

Photosynthesis assessment using Imaging-PAM fluorometry, which allows capturing two-dimensional images of Chl fluorescence parameters, revealed high spatial heterogeneity within the macroalgal thallus. Consistently higher F_o values and lower photosynthetic yields were observed at the tips of *C. tomentosum* thalli. This indicates a higher chloroplast abundance in these regions (higher F_o) that causes a canopy effect that may limit light absorption by chloroplasts in the basal portions of the algal thallus. Giovagnetti et al. (2018) reported that the siphonous green macroalga *Bryopsis corticulans* can cope with excess light energy by activating sustained protective nonphotochemical quenching in algal surface filaments. While this undermined photochemistry in surface filaments, it allowed efficient photosynthetic rates deeper in the algal structure (Giovagnetti et al. 2018).

Codium and other macroalgae within the Bryopsidales lack the xanthophyll cycle (Cruz et al. 2015; Christa et al. 2017), the most ubiquitous photoprotection mechanism present in most algae and higher plants. In this cycle, Viola is converted sequentially to antheraxanthin and zeaxanthin under high irradiance, with the latter pigment playing a crucial role as energy dissipator (Goss and Lepetit 2015). In *C. tomentosum*, Viola represented only 1.4% of carotenoid abundance and zeaxanthin was not detected. This could be a major drawback for an intertidal and shallow subtidal macroalgae exposed to extreme light fluctuations associated with the tidal cycle. An alternative xanthophyll cycle has been observed in higher plants involving the conversion of lutein–5,6–epoxide to lutein under high irradiance (Bungard et al. 1999; Jahns and Holzwarth 2012). However, these pigments were absent in *C. tomentosum* (Table 1). The accumulation of the pigment *trans*-neoxanthin has been

observed in *C. intricatum* and *C. tomentosum* acclimated to high irradiance (Uragami et al. 2014; Cartaxana et al. 2018), but a photoprotective role for *trans*-neoxanthin is yet to be established. Photoprotection in Bryopsidales is most likely provided by an array of different mechanisms that may include uncharacterized nonphotochemical quenching mechanisms, efficient D1 protein turnover and reactive oxygen species scavenging, and chloroplast streaming (Franklin and Larkum 1997; Christa et al. 2017; Giovagnetti et al. 2018).

Understanding the appropriate light requirements of *C. tomentosum* will assist and help to expand commercial cultivation of this macroalga. In addition, optimized cultivation conditions under IMTA systems will lower the costs associated to the treatment of excess of nutrients in the fish farm effluents to fulfill environmental regulations and enhance aquaculture revenues. We propose long-day photoperiod (LD, 16 h light:8 h of dark) and light sources with preferential emission at the red region of the light spectra as optimal for growing *C. tomentosum* at early development stages. These conditions can reduce the time required for the initial growth phase and anticipate the transfer to grow-out systems. Increasing *C. tomentosum* productivity will help satisfying the current high demand by the market for sustainably cultured biomass of this macroalga.

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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