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Journal of Applied Phycology
https://doi.org/10.1007/s10811-024-03317-8

RESEARCH

Photosynthetic physiology and antioxidant compounds in *Gracilaria cornea* (Rhodophyta) under light modulation

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Received: 7 March 2024 / Revised: 4 July 2024 / Accepted: 9 July 2024

Abstract

Gracilaria cornea was exposed to different irradiance intensities (low, moderate, and high) and light qualities (white and white + blue) in experimental cultures. Photosynthetic physiology was measured through daily growth rate, maximum photosynthesis, maximum photochemical efficiency, pigment content (chlorophyll *a*, phycobiliproteins, carotenoids), and elemental content of carbon and nitrogen. *Gracilaria cornea* effectively acclimated to low irradiance conditions and displayed tolerance to moderate irradiance, whereas high irradiance resulted in the deterioration of thalli. A decrease in maximum photochemical efficiency and maximum photosynthesis rate under high irradiance conditions was observed. Pigment content decreased during the acclimation period at high irradiance levels but increased when blue light was added under moderate irradiance conditions. The antioxidant capacity decreased in thalli exposed to high irradiance conditions, whereas the addition of blue light increased antioxidant capacity. Overall, the combination of white and blue light stimulated the accumulation of all evaluated compounds in *G. cornea*. Interestingly, higher values for photosynthesis, pigments, and certain antioxidants were observed under low irradiance conditions. These findings enhance our understanding of the adaptation strategies employed by *G. cornea*, potentially leading to improvements in indoor cultivation and the control of chemical compound production for nutraceutical applications.

Keywords *Gracilaria* · Irradiance · Light quality · Photosynthetic response · Antioxidants · Nutraceuticals

Introduction

The availability of *Gracilaria cornea* along the north coast of Yucatán, Mexico, has made it a promising candidate for commercial applications (Orduña-Rojas et al. 2002a, b). Moreover, extensive investigations have explored its potential as food and a valuable source of agar (Freile-Pelegrín and Robledo 1997; Freile-Pelegrín et al. 2002; Pereira-Pacheco et al. 2007). Therefore, *G. cornea* has emerged as an ideal species for cultivation. Extracts derived from this macroalga exhibit remarkable bioactive properties including antioxidant, anti-inflammatory, antitumor, and immunological activities, making it an attractive subject of study (Zubia et al. 2007; Coura et al. 2012; Álvarez-Gómez et al. 2019).

Metabolite production in algae is governed by various physicochemical variables, with light being the most relevant. Irradiance and light quality in the ocean are primarily regulated by depth; however, they are also influenced by other factors, such as turbidity and the intrinsic properties of water. Algae adjust their metabolic activities, optimize photosynthesis during daylight hours, and transition to other processes such as energy storage during the photoperiod, marked by alternating light and dark phases (Hurd et al. 2014). During these processes, algae synthesize a wide variety of chemical compounds, each with distinct bioactive properties. This includes pigments, such as carotenoids, phycobiliproteins, phenols, and certain polysaccharides (Blunt et al. 2007).

Irradiance plays a pivotal role in regulating photosynthesis and other important physiological responses in algae, directly influencing growth, productivity, and overall ecological success (Kameyama et al. 2021; Terada et al.

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Published online: 15 August 2024

2021). Elevated irradiance typically engenders heightened maximum photosynthesis rates up to an apparent threshold, after which photosynthetic performance reaches a plateau, emphasizing the finite capacity of macroalgae to utilize incident light. However, excessive light can lead to the inhibition of photosynthesis through photodamage due to the generation of oxidizing species. Reactive oxygen species (ROS) reduce photosynthesis activity by oxidizing proteins and nucleic acids, and degrading chlorophyll *a* (Bischof and Rautenberger 2012). Furthermore, nuances in light quality, encompassing the spectral composition of incident light, hold sway over the maximum photosynthesis rates. Macroalgal species may acclimate to specific wavelengths, thereby modulating their photosynthetic potential across varying light environments (Borlongan et al. 2020; Shindo et al. 2022).

Macroalgae exhibit distinct daily growth rate (DGR) patterns in response to fluctuating irradiance and light quality. Elevated irradiance often triggers an augmentation in DGR, as the increased availability of photons stimulates photosynthetic activity. Nonetheless, this response is nuanced, and species-dependent, as excessively high irradiance can induce photoinhibition, leading to a decrease in DGR owing to potential damage to the photosynthetic apparatus (Singh and Singh 2015; Marques et al. 2021). Additionally, assessment of the maximum photochemical efficiency of photosystem II (PSII) F_v/F_m in macroalgae offers a sensitive gauge of their photosynthetic robustness. This metric quantifies the maximum efficiency with which macroalgae convert absorbed light into chemical energy via photosynthesis, thereby providing valuable insights into their photosynthetic "health status" (Büchel and Wilhelm 1993). Excessive irradiance or imbalances in the spectral composition can lead to a decline in F_v/F_m, signifying potential PSII impairment and diminished photosynthetic efficiency in macroalgae (Wu et al. 2015; Wu 2016).

The regulation of pigment synthesis and other biochemical compounds in macroalgae is also affected by irradiance and light quality. Changes in the spectral composition of light, driven by variables such as water depth, dissolved and particulate substances, and daily or seasonal climate shifts, influence algal photosynthesis through their effects on photomorphogenesis and photosynthetic pigment composition (Hurd et al. 2014). These pigments play a relevant role within photosystems by actively regulating algal photosynthetic efficiency to align with the prevailing light spectrum (Larkum 2003). For example, carotenoids can donate electrons, thereby reducing the adverse effects of free radicals (Freile-Pelegrín and Robledo 2013). Additionally, phycobilisomes function as photosynthetic antennae, and it has been demonstrated that the pigments within them, such as phycoerythrin and phycocyanin, possess antioxidant capacities (Pagels et al. 2019). Polyphenols, such as phlorotannins, have also been found to exhibit antioxidant activities in certain red macroalgae (Kim et al. 2005).

Irradiance and light quality also play crucial roles in modulating various aspects of macroalgal physiology including photosynthetic activity, growth, cell density, and morphological characteristics (Carmona et al. 1998; Talarico and Maranzana 2000; Tsekos et al. 2002). Macroalgae experience dynamic and diverse temporal and spatial conditions in their natural environment. To adapt to these environmental fluctuations, macroalgae have developed specific strategies at different timescales, such as antioxidant production, arrangement of light-harvesting antennae, and synthesis of compounds such as polysaccharides (Zubia et al. 2014; Peñuela et al. 2018).

Red algae are known to produce phycocolloids such as agar in response to environmental stress. Agar yield in *G. cornea* from Yucatán is strongly affected by surrounding environmental conditions (Freile-Pelegrín and Robledo 1997). Light quality also has an impact on polysaccharide production. Furthermore, they found that blue light promotes the synthesis of galactans in this species. Algal polysaccharides exhibit bioactive properties, including notable antioxidant and antiviral effects. Combining agars from certain *Gracilaria* species with other compounds has demonstrated antioxidant capacity (Rodríguez-Félix et al. 2017; Roy and Rhim 2019). Exploring the properties of these complex carbohydrates not only enhances our understanding of the antioxidant potential of algae species but also provides insights into the mechanisms employed by these organisms to adapt to their diverse habitats.

Recent research has yielded promising results in *G. cornea*, demonstrating that growth rate and carotenoid and phycobiliprotein levels can be enhanced by manipulating the light quality in laboratory cultures, particularly with the addition of blue light (Zepeda et al. 2020). However, no study has examined the effect of irradiance on the production of antioxidant compounds in this alga. The antioxidant capacity of algae is often associated with the synthesis of phycobiliproteins, carotenoids, polyphenols, and other molecules. By evaluating the algal extracts in conjunction with metabolite data, we gained insights into the ability of the studied macroalgae to counteract oxidative stress caused by several factors. Based on previous findings, we aimed to assess and characterize the physiological, biochemical, and antioxidant changes in *G. cornea* as a function of distinct levels of irradiance and blue light addition. This information will help improve our understanding of the regulation of antioxidant metabolites, contribute to the existing literature, and enhance the potential application of *G. cornea* compounds in the pharmaceutical and cosmetic industries.

Materials and methods

Material collection and experimental design

Thalli fragments of *Gracilaria cornea* were collected in October 2020 from the Cinvestav Telchac Marine Station. These algae were grown in outdoor 1500 L tanks covered with shade mesh (~80% light attenuation), a flow-through system ensuring total water replacement without nutrient addition and continuous aeration. During this season, the outdoor cultures exhibited salinity and temperature ranges of 32–34 and 28–32 °C, respectively. Photosynthetically active radiation (PAR) at the water surface ranged between 360 and 500 µmol photons m^{-2} s^{-1} at noon. Following collection, the biological material was carefully transported to the laboratory and thoroughly rinsed with sterile seawater to remove any potential contaminants, including epiphytes. Subsequently, the algae were meticulously fragmented into thalli weighing approximately 3 g. After this, the fragments followed a sequential process of maintenance and acclimation to light, and thereafter were subjected to experimental treatments.

Maintenance of the experimental fragments was done in the laboratory for 10 days using 15-L cylindrical acrylic bioreactors with continuous aeration applied from the bottom of the bioreactors. Seawater changes were conducted weekly, without the addition of nutrients, with a salinity of 30, temperature of 21.5 ± 0.23 °C, and irradiance of 111.0 ± 22.04 µmol photons m^{-2} s^{-1} under a photoperiod of 12:12 light:dark. The light source for the bioreactors (Current Orbit IC Pro Dual LED lamps) had a cool white spectrum (400–700 nm, 6500–10000 K) with a blue peak at 450 nm (Fig. S1a) and was sides of the bioreactors. The lamp system consists of an array of LEDs that can be modulated to achieve different wavelengths. During maintenance, the maximum photochemical efficiency (F_v/F_m) of *G. cornea* fragments remained stable (0.5-0.6).

The experimental treatments included a reference biomass treatment kept under cool white light at 111.0 ± 22.04 µmol photons m^{-2} s^{-1} with nutrient addition, hereafter referred to as control. The experimental treatments consisted of two different irradiance levels: moderate (ML) with an intensity of 325.1 ± 20.24 µmol photons m^{-2} s^{-1} and high (HL) with an intensity of 646.3 ± 22.89 µmol photons m^{-2} s^{-1}. The samples were maintained at a density of approximately 5 g L^{-1} in the bioreactors used for each treatment. To increase light reflection and provide homogeneous illumination within the bioreactors these were covered with aluminum foil (Fig. 1a). In the first week, only cool white light (+W) was provided, and during the second week, we used a combination of cool white and blue light (+WB) for the experimental treatments (ML and HL) by adjusting the quality of the LEDs (Fig. 1b). The cool white light covered a spectrum from 400 to 700 nm, whereas the blue light had an emission peak between 445 and 460 nm (Fig. S1b). In this arrangement, there was a greater proportion of blue light than cool white light alone, specifically at a ratio of 2:1 (blue light to cool white light). Lamp irradiance was measured using a universal light meter (WALZ ULM-500) connected to a microspherical sensor (US-SQS). Four bioreactors were used for each treatment.

Temperature and salinity were measured daily and kept within 21.7 ± 1.28 °C and 31.7 ± 0.89, respectively. Enriched sterile seawater (Provasoli 1968) was renewed weekly to prevent epiphyte growth and nitrogen deficiency in algal tissue. *Gracilaria cornea* thalli (Fig. 1c) were harvested from each replicate on day 7 (1st week) and 14 (2nd week), rinsed with distilled water to remove salts, weighed, and either frozen or lyophilized, depending on the analytical protocol. After harvesting, culture densities in each cylinder were reset to 5 g L^{-1}.

Algal physiological measurements

Gracilaria cornea daily growth rate (DGR) was calculated weekly using the equation described by Yong et al. (2013). The maximum photochemical efficiency (F_v/F_m) was obtained using a portable fluorometer (PEA; Hansatech, UK) coupled with a clip for sample placement. Measurements were taken 30 min after the samples were placed in total darkness, followed by saturating with red light pulses (3000 µmol photons m^{-2} s^{-1} for 1 s). The F_v/F_m value was measured in five thalli from each replicate on days 0, 7, and 14.

The influence of irradiance on photosynthetic processes in algae is of great significance therefore we evaluated a photosynthesis-irradiance (P-E) curve in control organisms before initiating the light experiments to understand how *G. cornea* responds to distinct levels of irradiance and was used as a reference to set irradiance for the experimental photosynthetic descriptors. Fragments of the apical region weighing between 100 and 200 mg were cut from the initial biological material (time 0, ~100 µmol photons m^{-2} s^{-1}) 1 h before the measurements. These fragments were then incubated in the dark for 10 min to measure dark respiration. Oxygen evolution (gross photosynthesis, GPS) was quantified along an irradiance gradient ranging from 25 to 1500 µmol photons m^{-2} s^{-1}, every 5-6 min at each irradiance level. Subsequently, the samples were returned to darkness for 10 min to measure the post-illumination respiration (Vásquez-Elizondo and Enríquez 2016). Measurements were performed using a Clark-type oxygen polarographic electrode connected to a respirometry system (DW3-Oxygraph Hansatech, UK). The respirometry chamber, containing the thalli suspended in approximately 15 mL of sterile seawater

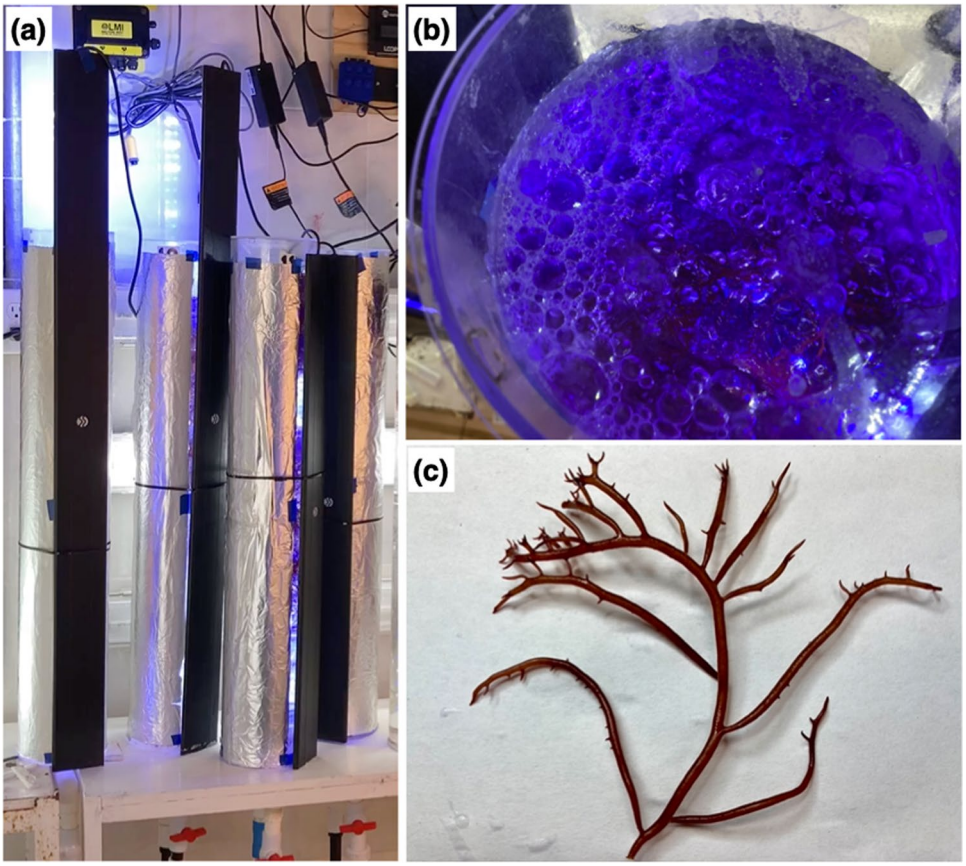

Fig. 1 Experimental setup. (**a**) Cylindrical acrylic bioreactors (15 L) with an array of LED lamps positioned at the side; (**b**) A top view of the bioreactors shows *Gracilaria cornea* movement facilitated by air at the bottom of the cylinder; (**c**) Details on the specimen of *G. cornea* used in the experiment

and illumination, was provided with a Hansatech lamp (LH36/2R LED) connected to a circulating water bath to provide a constant temperature of 22 °C. To prevent carbon limitation, sodium bicarbonate ($NaHCO_3$, 4.5 µM) was added to the medium. Maximum photosynthesis (P_{max}), initial slope (α), dark respiration (R_D), light respiration (R_L), and saturation light index (E_k) were determined using the P-E curve following Vásquez-Elizondo and Enríquez (2016). The P_{max}, R_D, and R_L values were normalized based on the fresh weight (Table S1).

Under the experimental treatments (ML and HL), oxygen production reported as gross photosynthesis in *G. cornea* was evaluated as described above to gain insight into the physiological response. This assessment provided photosynthetic performance specifically P_{max} and R_L for all treatments at 648 µmol photons m^{-2} s^{-1} irradiance used based on the P-E curve for the control. This approach was chosen because we observed a stable P_{max} at that irradiance in organisms exposed to control conditions (Fig. S2).

Pigment content analysis and C:N ratio

Samples were extracted for pigment analysis, specifically for chlorophyll *a* (Chl *a*) and total carotenoids (Car) using acetone and phycoerythrin (Pe) using phosphate buffer. In brief, 100 mg of fresh algae was collected from the apical region of *G. cornea* thalli for acetonic extraction, 1 g of fresh weight was used for sodium phosphate buffer extraction. The samples were pulverized in liquid nitrogen using a mortar and pestle. All extractions were carried out in the dark, followed by centrifugation at 1700 × *g* for 15 min at 10 °C to collect the supernatant. The absorbance was measured using a Shimadzu UV-1800 spectrophotometer (Shimadzu, Japan). Pigment concentrations were calculated using the following established formulae (Dawes 1998):

$$\text{Chl } a = 11.9\left(A_{663}\right)(av)/((\text{FW})(\%\text{DW})(1000))$$
$$\text{Car} = 7.6\left(A_{480} - \left(1.49\left(A_{510}\right)\right)\right)(av)\ /\ ((\text{FW})(\%\text{DW})(1000))$$
$$\text{Pe} = 12.4\left(A_{565}\right)(bv)\ /\ ((\text{FW})(\%\text{DW})(1000))$$

where absorption coefficients were expressed in mg L^{-1}, *av* and *bv* refer to the volumes of acetone and phosphate buffer in mL, respectively. FW corresponds to fresh weight (g) and %DW refers to the dry weight to fresh weight ratio. Data are expressed as mg g^{-1} DW. Thereafter, the chlorophyll *a* to phycoerythrin (Chl *a*:Pe) ratio was calculated for each experimental condition.

High-performance liquid chromatography (HPLC) was used to complement spectrophotometric measurement analysis to identify and quantify carotenoids. Lyophilized samples

(20 mg) were extracted using a mixture of 75% acetone and 0.5% acetic acid at a concentration of 80 mg mL^{-1} (Prior et al. 2010). The extracts were sonicated in an ice bath for 5 min and stirred at 700 rpm at room temperature (20 °C) for 1 h. Subsequently, the samples were centrifuged at 110 × *g* for 10 min and the supernatant was transferred to an HPLC vial. HPLC analysis was performed using a Dionex UltiMate 3000 UHPLC system (Thermo Scientific) according to a modified protocol (Silkina et al. 2009). Carotenoids were separated using a NUCLEODUR 100-5 C18ec column (250 x 4.6 mm, 5 µm; Macherey-Nagel) with a guard column. A 40-min run was conducted with a flow rate of 1.4 mL min^{-1} and a solvent gradient (Table S2). Identification of each carotenoid pigment was based on spectral and time comparisons using commercial standards of astaxanthin (Axt), lutein (Lut), α-carotene (α-car) (Sigma Aldrich, USA), β-carotene (β-car), and zeaxanthin (Zxt) (DHI, Denmark). The data were expressed in µg g^{-1} DW and were collected using Dionex Chromeleon 7.2.10 software (Thermo Scientific).

Carbon (C) and nitrogen (N) contents were analyzed weekly by the complete combustion of 10 mg dry weight (DW) samples using a FlashEA 1112 analyzer (Thermo, Italy). Values are expressed as mg g^{-1} DW.

Total phenolic content and antioxidant capacity

Polyphenols are crucial components of the antioxidant capacity in algae, making their quantification essential for understanding the bioactive properties of *G. cornea* exposed to experimental treatments. Polyphenol extractions used methanol (100%) in 2 g of fresh weight samples. The methanolic extracts were evaporated using a vacuum rotavapor (Buchi, Switzerland) and then resuspended in methanol (5 mg mL^{-1}). Total polyphenols were quantified using the Folin-Ciocalteu assay adapted for microplates (Zhang et al. 2006). In this assay, 20 µL of the methanol extract was mixed with 100 µL of Folin-Ciocalteu reagent and 80 µL of 7.5% sodium carbonate (Na_2CO_3). The mixture was vortexed and incubated in the dark for 2 h. The absorbance was measured at 750 nm using a Multiskan GO UV/Vis spectrophotometer (Thermo Fisher Scientific, USA). To calculate the sample concentrations, a calibration curve was constructed using phloroglucinol in the concentration range of 6.25 to 100 µg mL^{-1}. The total phenolic content (TPC) is expressed as percentage of phloroglucinol equivalents (%PGE).

To assess the antioxidant capacity, methanolic extracts (phenols) and phosphate buffer extracts (phycobiliproteins) were subjected to modified 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) with some adaptations and ferric reducing antioxidant power (FRAP) protocols. These protocols were adapted for use with 96-well microplates, and measurements were taken using a spectrophotometer as previously described (Fukumoto and Mazza 2000; Arnao et al. 2001; Turkmen et al. 2007; Zubia et al. 2009; Kenny et al. 2015). The use of various methodologies allows the integration of the information obtained from each protocol. Certain procedures uncover the antioxidant capacity of chemical compounds in crude extracts, which dissolve in water, whereas others are used in organic solvents. This approach enables a thorough and complete comprehension of the antioxidant responses (Sadeer et al. 2020).

For the DPPH assay a 0.25 mM solution was prepared in 100% methanol. From this solution, 180 µL was combined with 20 µL of the sample in each well of the microplate. The plate was then placed in the dark for 1 h, and the absorbance was measured at 515 nm. For the ABTS assay, a 7 mM ABTS reagent solution was prepared by adding 2.45 mM potassium persulfate prior to the reaction. Subsequently, 200 µL of ABTS solution was mixed with 20 µL of the sample in each well of a microplate. The reaction mixture was incubated in the dark for 10 min and the absorbance was measured at 734 nm. DPPH and ABTS results were expressed as a percent of scavenging activity.

For the FRAP protocol a stock FRAP solution was prepared with acetate buffer, ferric chloride, and 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ). A calibration curve was constructed using Trolox at concentrations ranging from 0 µM to 480 µM. To perform the reaction, 25 µL of the sample was mixed with 175 µL of FRAP solution in each well of the microplate. Methanol and distilled water were used as blanks for background correction. The microplate was then incubated at 37 °C for 40 min. After incubation, the absorbance of the reaction mixture was measured at 593 nm. The antioxidant capacity results obtained from the FRAP assay were expressed as micromoles of Trolox equivalents per gram of dry weight (µM TE g^{-1} DW), which served as a standard for comparison. Methanol was used as a negative control, whereas butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) were used as positive controls. In addition to the samples, the antioxidant capacity was also evaluated using commercial standards of Chl *a* (Bio-Chemika), R-phycoerythrin, and C-phycocyanin (Sigma Aldrich), as mentioned before.

Agar extraction

Experimental samples were also used for the extraction of native agar by the microwave-assisted extraction (MAE) technique using 1 g of dried and ground algae per treatment (Vázquez-Delfín et al. 2014). The samples were hydrated with distilled water at room temperature and transferred to a sealed reaction vessel (OMNI/Xpress-1500) to prevent any loss of the sample or water. The extraction process was carried out using an accelerated reaction microwave system

(MARS 800 W, CEM, Matthews, USA) at 100 °C for 20 min. The temperature and pressure (159 kPa) were closely monitored throughout the extraction process. Following extraction, the agar was allowed to thaw at room temperature, excess water was drained, and the obtained material was dried in an oven at 60 °C for one day. Subsequently, the dried agar was placed in a desiccator, allowed to equilibrate for one hour, and weighed to determine the yield (%), which was calculated based on the initial and final weights of the samples. The analysis of 3,6 anhydro-galactose (3,6 AG) and sulfate content (SO_4^{2-}) in agar extracts followed established procedures (Jackson and McCandless 1978; Matsuhiro and Zanlungo 1983). The content of both 3,6 AG and SO_4^{2-} was expressed as a percentage.

Statistical analysis

One-way ANOVAs (analysis of variance) were used to assess differences in all variables. Before conducting the ANOVA analyses, tests for homogeneity of variances (Levene) and normality (Shapiro-Wilk) were performed. If the variables did not meet the assumption of normality, log10 transformations were applied. Significant differences between treatments were examined using the post-hoc Tukey's test ($p < 0.05$). Pearson product-moment correlations were calculated for all evaluated variables. Statistical tests and analyses were conducted using the statistical program Jamovi version 2.3 (The Jamovi project 2023).

Results

Algal physiology

The daily growth rate of *G. cornea* did not show significant variation among the experimental light treatments, ranging from 1.6 to 2.2% day^{-1}. Likewise, the F_v/F_m values throughout the experiment did not change significantly and were in the range of 0.4 to 0.5. However, we found significant differences in the P_{max} values (Fig. 2a, ANOVA: $F_{4,4.3} = 19.9$, $p = 0.005$), specifically during blue light addition (Table 1). For the ML treatment, P_{max} increased during the second week, whereas in the HL treatment, a decrease was observed. Post-illumination respiration (R_L) was influenced by light conditions (Fig. 2b, ANOVA: $F_{4,4.6} = 16.4$, $p = 0.006$). Over time, R_L decreased in comparison with the control, except in the HL+WB group.

Pigment content

Experimental treatments showed significant differences (ANOVA: $F_{4,4.8} = 26.2$, $p = 0.002$) in the Chl *a* content of *G. cornea*. In most treatments, Chl *a* values were lower than those of the control, except for ML+WB, where it remained unchanged (Fig. 2c). Notably, an increase in Chl *a* content was observed in the ML treatment during the second week following the addition of blue light. Regarding the synthesis of Car, the results had a similar effect (ANOVA: $F_{4,4.5} = 18.2$, $p = 0.005$) as observed for Chl *a*. In the HL treatment, a decrease in Car values was found compared to the control, whereas no significant differences were observed in the ML condition (Fig. 2d). HPLC analyses identified the presence of Zxt and β-car in the samples however, Zxt was only detected under high light exposure (0.3 ± 0.15 to 0.4 ± 0.09 µg g^{-1} DW), while β-car was synthesized exclusively in ML+W (5.3 ± 1.01 µg g^{-1} DW). In the HPLC chromatograms of the remaining samples, the peaks were below the detection limit.

The Pe content decreased in all experimental treatments. The highest value was observed in ML+WB during the second week when blue light was added (Fig. 2e). Light conditions had a significant effect on the production of Pe (ANOVA: $F_{4,4.2} = 75.9$, $p < 0.001$) and Pc (ANOVA: $F_{4,4.6} = 11.5$, $p = 0.013$) in *G. cornea*. The Chl *a*:Pe ratio exhibited significant changes only in ML+W (Fig. 2f, ANOVA: $F_{4,4.2} = 6.34$, $p = 0.046$). Notably, a decrease in the Chl *a*:Pe ratio was observed in the ML treatment when blue light was used. Furthermore, a significant positive correlation (Table 2, Pearson's correlation coefficient: r = 0.7-0.8, $p < 0.001$) was found between all evaluated pigments and P_{max}.

Carbon and nitrogen content

Carbon content did not show significant differences among experimental conditions; values ranged between 308 ± 19.6 and 337 ± 6.2 mg g^{-1} DW. However, nitrogen values ranged between 26 ± 1.0 and 38 ± 1.2 mg g^{-1} DW (ANOVA: $F_{4,6.6} = 40.6$, $p < 0.001$) and C:N ratio (ANOVA: $F_{4,6.8} = 49.9$, $p < 0.001$) exhibited significant differences among treatments. The nitrogen content in *G. cornea* increased when blue light was added at moderate irradiance (Fig. 2g), which was consistent with the observed trend in Pe synthesis. Specifically, a decrease in the C:N ratio was observed in ML+WB, whereas in the other light conditions, the values remained the same as in the control (Fig. 2h).

Total phenolic content and antioxidant capacity

Light conditions did not have a significant effect on the polyphenol content of *G. cornea*. The TPC ranged from 0.12 ± 0.00 to 0.23 ± 0.05 %PGE. In general, low irradiance (control) stimulated higher antioxidant activity in the methanolic extracts of *G. cornea* in all the protocols used (Fig. 3a-c). Significant differences (ANOVA: $F_{4,4.1} = 65.6$, p

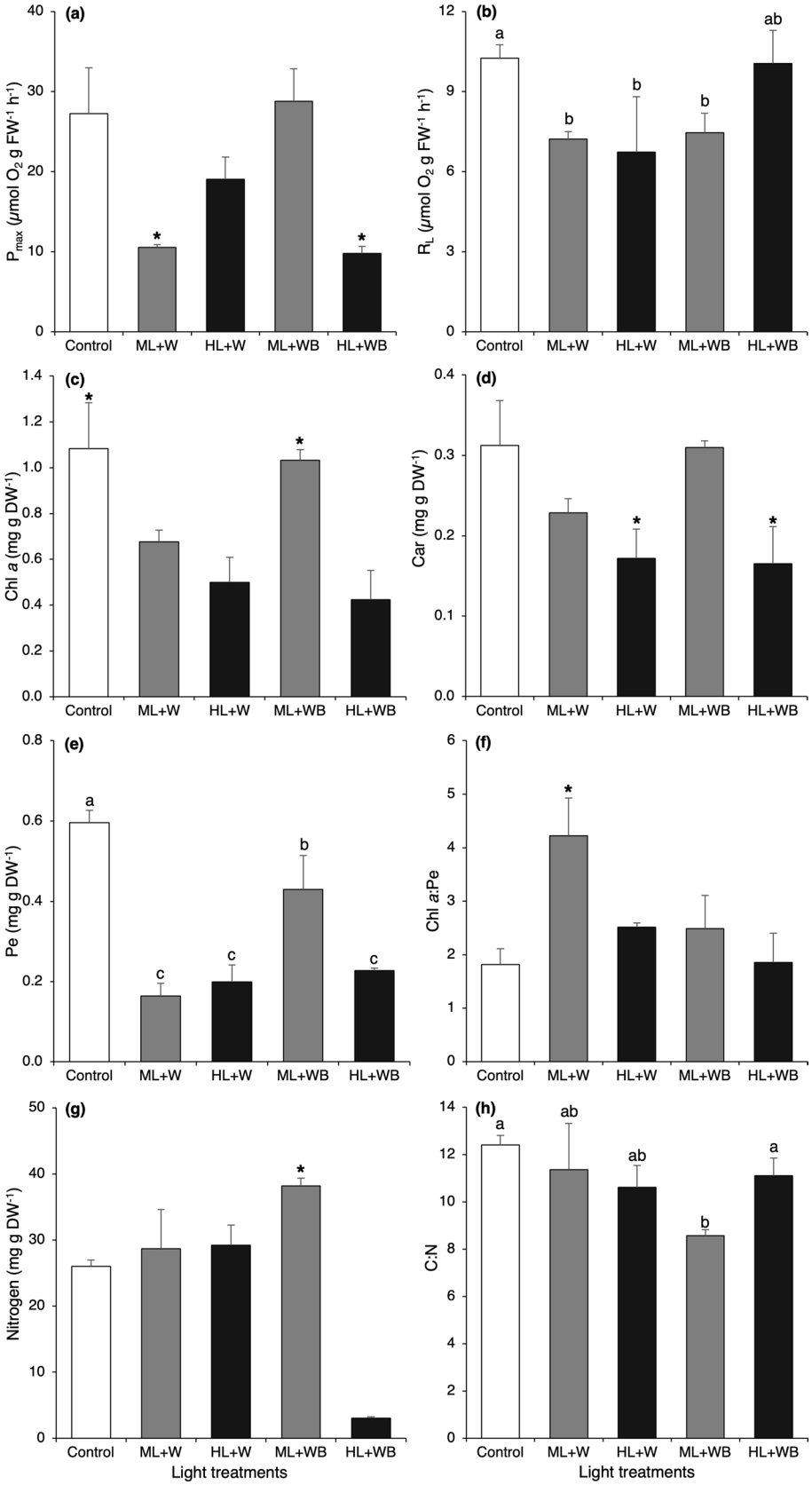

Fig. 2 Physiological parameters of *Gracilaria cornea* exposed to 100 µmol photons m^{-2} s^{-1} (control); 350 µmol photons m^{-2} s^{-1} with white light (ML+W) and 700 µmol photons m^{-2} s^{-1} with white light (HL+W) during first week; 350 µmol photons m^{-2} s^{-1} with white and blue light (ML+WB) and 700 µmol photons m^{-2} s^{-1} with white and blue light (HL+WB) during the second week. (**a**) Maximum photosynthesis; (**b**) Post-illumination respiration; (**c**) Chlorophyll *a*; (**d**) Total carotenoids; (**e**) Phycoerythrin; (**f**) Chlorophyll *a*:Phycoerythrin ratio; (**g**) Nitrogen; (**h**) Carbon:Nitrogen ratio. Bars represent the mean ± standard deviation ($n = 3$). Significant differences among treatments are indicated by asterisks and different letters (ANOVA, $p < 0.05$)

Table 1 ANOVA results of experimental variables in *Gracilaria cornea*: Maximum photochemical efficiency (F_v/F_m), maximum photosynthesis (P_{max}), light respiration (R_L), chlorophyll *a* (Chl *a*), total carotenoids (Car), phycoerythrin (Pe), phycocyanin (Pc), Chl *a*:Pe ratio, carbon (C), nitrogen (N), C:N ratio, total phenolic content (TPC), antioxidant capacity of methanolic (DPPH, ABTS, FRAP Met) and phycobiliprotein extracts (DPPH, ABTS, FRAP PBP), agar, 3,6 anhydro-galactose (3,6 AG) and sulfate content (SO_4^{2-}). Significant values ($p < 0.05$) are indicated in bold

Variable*	*F*	d. f	*p*
F_v/F_m	0.35	4,22	0.840
P_{max}	19.90	4,4.3	**0.005**
R_L	16.40	4,4.6	**0.006**
Chl *a*	26.20	4,4.8	**0.002**
Car	18.20	4,4.5	**0.005**
Pe	75.90	4,4.2	**< 0.001**
Pc	11.50	4,4.6	**0.013**
Chl *a*:Pe	6.34	4,4.2	**0.046**
C	2.68	4,6.2	0.132
N	40.6	4,6.6	**< 0.001**
C:N	49.9	4,6.8	**< 0.001**
TPC	3.02	4,4	0.154
DPPH Met	6.26	4,4.2	**0.047**
ABTS Met	65.6	4,4.1	**< 0.001**
FRAP Met	5.75	4,4.9	**0.043**
DPPH PBP	26.9	3,4.3	**0.003**
ABTS PBP	84.1	4,4.4	**< 0.001**
FRAP PBP	231	4,4.6	**< 0.001**
Agar	123	3,3.6	**< 0.001**
3,6 AG	0.15	3, 4.4	0.927
SO_4^{2-}	2.92	3,4.2	0.158

**F* Fisher test, *d. f.* degree of freedom, *p* probability

< 0.001) were found among the experimental treatments for the methanolic extracts evaluated using the ABTS method. The DPPH (ANOVA: $F_{4,4.2} = 6.26$, $p = 0.047$) and FRAP (ANOVA: $F_{4,4.9} = 5.75$, $p = 0.043$) methods showed differences only for different light conditions exposure (ML *vs* HL). There was a positive correlation between the polyphenol content and antioxidant capacity of the methanolic extract evaluated by the FRAP method (Pearson's correlation coefficient: r = 0.73, $p = 0.002$). In addition, Chl *a* and Car showed a significant correlation with the methanolic antioxidant activity of DPPH (Pearson's correlation coefficient: r = 0.71, $p = 0.003$ and r = 0.67, $p = 0.007$, respectively).

The phosphate buffer extract of *G. cornea* showed higher antioxidant activity using the DPPH method when exposed to moderate irradiance (ML+W). This treatment resulted in an increase in antioxidant activity compared to that of the control during the first week. However, in the second week, a decrease in antioxidant activity was observed under white and blue light conditions (Fig. 3d). Unfortunately, we were unable to recover HL+W data for the DPPH protocol because of sample loss. In contrast, in the ABTS assay, the antioxidant activity values were higher when *G. cornea* was exposed to high light conditions (HL+WB), but there were no significant changes compared with the control (Fig. 3e).

The antioxidant capacity obtained with the FRAP method was higher when *G. cornea* was exposed to ML+WB. Only the treatments irradiated with blue light showed higher antioxidant activity than that of the control (Fig. 3f). Light conditions had a significant effect on the ABTS (ANOVA: $F_{4,4.4} = 84.1$, $p < 0.001$) and FRAP results (ANOVA: $F_{4,4.6} = 231$, $p < 0.001$). The antioxidant activity of *G. cornea* phosphate buffer extract was generally higher under moderate irradiance (350 µmol photons m^{-2} s^{-1}). Additionally, the antioxidant capacity of the methanolic extracts of *G. cornea* was only half that observed in the Chl *a* standard with DPPH. However, in the phosphate buffer extracts, the values were comparable to those obtained with ABTS in the Pe and Pc standards but significantly higher than those obtained with FRAP (Table S3).

Polysaccharide chemical analysis

The content of polysaccharides was influenced by different light conditions (Fig. 4). Agar content showed significant differences between treatments (ANOVA: $F_{3,3.6} = 123$, $p <$ 0.001). Although the highest agar content was observed in the samples under control conditions, the effect of light quality enhanced the production of this phycocolloid under medium irradiance (ML) during the last week of cultivation. In contrast, the percentage of 3,6 AG and sulfate content did not exhibit significant differences among the treatments in *G. cornea*.

Discussion

Algal physiology and photosynthesis

In this study we used an experimental design to evaluate antioxidant compounds in *G. cornea* under different light treatments. The strategies employed by *G. cornea* involved their physiological acclimation to different light conditions. The duration and mechanisms of acclimation in algae vary depending on their initial physiological state, making this process stressful or non-stressful under different culture conditions. We noted swift acclimation of the organisms throughout both the maintenance and experimental weeks, despite increased irradiance. This suggests that it can endure moderate light intensities over time, making it a suitable candidate for cultivation to generate biomass or to produce a specific metabolite of interest.

Table 2 Pearson correlation matrix of experimental variables in *Gracilaria cornea*: Maximum photochemical efficiency (F_v/F_m), maximum photosynthesis (P_{max}), light respiration (R_L), chlorophyll *a* (Chl *a*), total carotenoids (Car), phycoerythrin (Pe), phycocyanin (Pc), Chl *a*:Pe ratio, carbon (C), nitrogen (N), C:N ratio, total phenolic content (TPC), antioxidant capacity of methanolic (DPPH, ABTS, FRAP Met) and phycobiliprotein extracts (DPPH, ABTS, FRAP PBP), agar, 3,6 anhydro-galactose (3,6 AG) and sulfate content (SO_4^{2-})

	F_v/F_m	P_{max}	R_L	Chl*a*	Car	Pe	Pc	Chl*a*: Pe	C	N	C:N	TPC	DPPH Met	ABTS Met	FRAP Met	DPPH PBP	ABTS PBP	FRAP PBP	Agar	3,6 AG	SO_4^{2-}
F_v/F_m	—																				
P_{max}	-0.12	—																			
R_L	0.24	-0.02	—																		
Chl*a*	-0.51	**0.82***	-0.12	—																	
Car	**-0.54**	**0.77**	-0.14	**0.99**	—																
Pe	-0.39	**0.78**	-0.48	**0.82**	**0.78**	—															
Pc	-0.25	**0.76**	-0.45	**0.71**	**0.68**	**0.87**	—														
Chl*a*:Pe	-0.02	-0.31	**0.57**	-0.03	0.00	**-0.55**	-0.46	—													
C	-0.26	-0.04	-0.09	-0.13	-0.15	0	-0.27	-0.42	—												
N	-0.20	0.34	0.09	0.23	0.26	0.18	0.00	-0.28	**0.57**	—											
C:N	-0.17	-0.15	**-0.62**	-0.04	-0.08	0.24	0.28	-0.26	-0.21	**-0.62**	—										
TPC	0.12	0.31	0.06	0.23	0.17	0.46	0.29	-0.29	-0.19	-0.07	0.17	—									
DPPH Met	**-0.52**	**0.66**	-0.39	**0.71**	**0.67**	**0.85**	**0.64**	-0.45	0.23	0.03	0.34	0.36	—								
ABTS Met	-0.30	0.44	**-0.61**	**0.52**	0.47	**0.77**	**0.76**	-0.42	-0.19	-0.35	**0.69**	0.38	**0.74**	—							
FRAP Met	0.09	0.31	-0.19	0.34	0.26	**0.56**	0.45	-0.26	-0.38	-0.50	**0.54**	**0.73**	**0.54**	**0.73**	—						
DPPH PBP	-0.00	0.13	0.53	0.36	0.37	-0.06	0.06	**0.70**	**-0.75**	-0.31	-0.11	0.26	-0.17	-0.02	0.16	—					
ABTS PBP	-0.45	0.00	**-0.88**	0.11	0.12	0.44	0.34	**-0.61**	0.42	0.13	0.49	-0.18	0.47	0.50	0.03	**-0.86**	—				
FRAP PBP	-0.31	0.48	-0.24	0.44	0.45	0.49	0.29	-0.43	**0.57**	**0.87**	-0.43	-0.07	0.33	-0.01	-0.29	-0.46	0.44	—			
Agar	-0.23	**0.76**	**-0.74**	0.67	**0.60**	**0.92**	**0.85**	-0.81	0.17	0.02	0.43	0.40	**0.84**	**0.88**	**0.67**	**-0.72**	**0.82**	0.38	—		
3,6 AG	-0.14	-0.08	0.09	-0.03	0.04	-0.07	0.01	-0.07	-0.06	0.02	-0.10	-0.14	-0.12	-0.25	-0.25	0.24	-0.21	-0.17	-0.19	—	
SO_4^{2-}	0.03	0.43	-0.01	0.35	0.39	0.33	0.25	-0.34	0.20	**0.78**	**-0.62**	0.08	-0.07	-0.24	-0.27	-0.41	0.11	**0.80**	0.11	0.08	—

*Significant correlations ($p < 0.05$) are indicated in bold

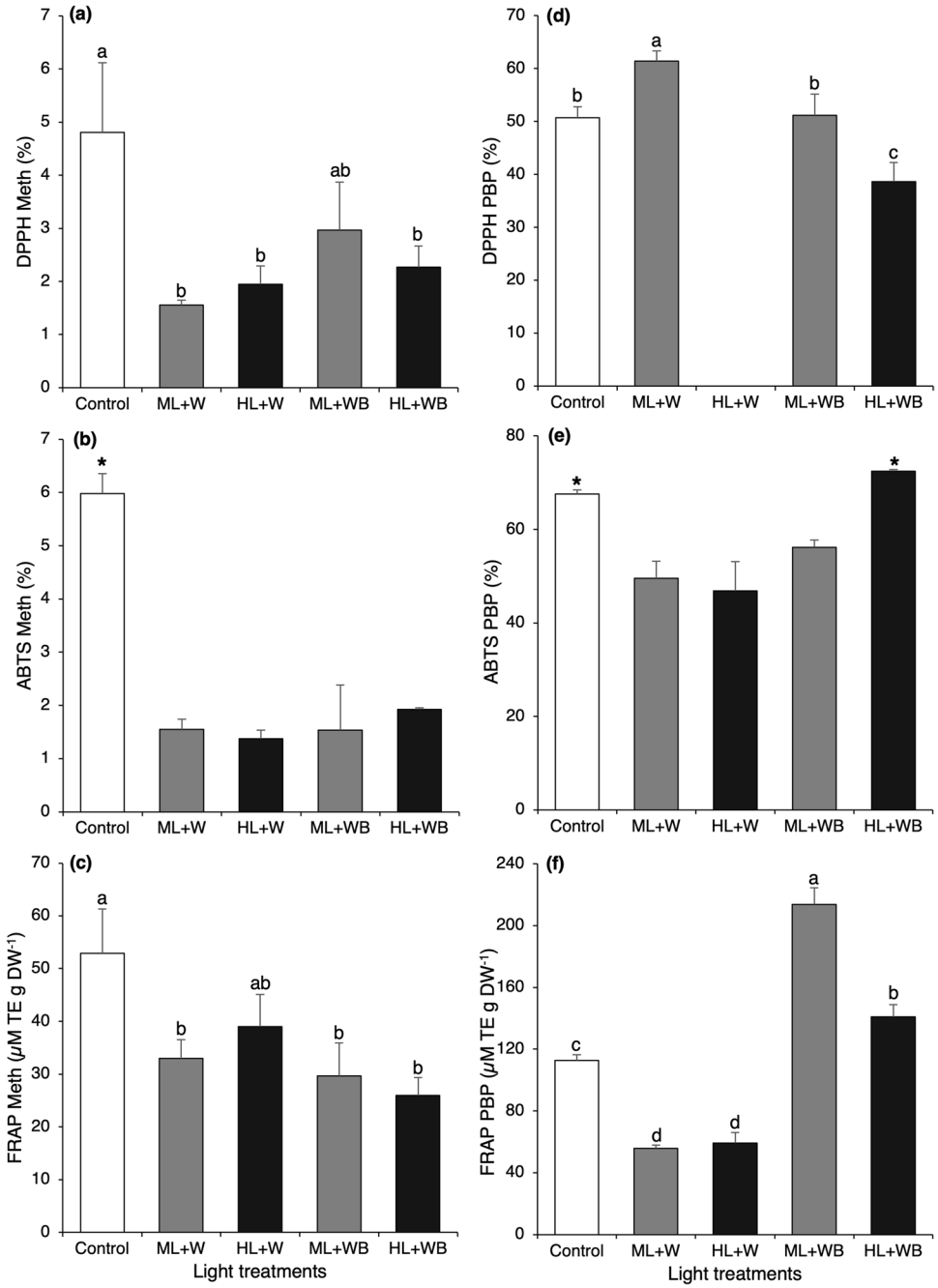

Fig. 3 Antioxidant capacity of *Gracilaria cornea* exposed to 100 µmol photons m^{-2} s^{-1} (control); 350 µmol photons m^{-2} s^{-1} with white light (ML+W) and 700 µmol photons m^{-2} s^{-1} with white light (HL+W) during first week 350 µmol photons m^{-2} s^{-1} with white and blue light (ML+WB) and 700 µmol photons m^{-2} s^{-1} with white and blue light (HL+WB) during the second week. (**a**) Percentage of DDPH scavenging activity of methanolic extracts; (b) Percentage of ABTS scavenging activity of methanolic extracts; (**c**) Antioxidant capacity of methanolic extracts evaluated by FRAP protocol; (**d**) Percentage of DDPH scavenging activity of phycobiliproteins extracts (HL+W data is missing due to sample loss); (**e**) Percentage of ABTS scavenging activity of phycobiliproteins extracts; (**f**) Antioxidant capacity of phycobiliproteins extracts evaluated by FRAP protocol. Bars represent the mean ± standard deviation ($n = 3$). Significant differences among treatments are indicated by asterisks and different letters ($p < 0.05$)

Daily growth rate, a relevant measure of performance, observed in *G. cornea* was consistent with that found in an initial study (Zepeda et al. 2020); however, Schneider et al. (2022) reported higher growth rates (%) in *G. cornea* exposed to irradiances ranging from 529 to 647 µmol photons m^{-2} s^{-1}. The consistently low growth of *G. cornea* observed during this study could be attributed to the energy expended in relocating plastids to minimize the incident PAR or to dissipate the excitation of pigments used in photosynthesis to counteract the damage resulting from exposure to high irradiance (Raven 2011). Additionally, this could be attributed to an effort to balance additional energy or metabolite synthesis to maintain scavenging mechanisms for the stabilization of growth. Otherwise, it might be indicative of a population successfully acclimated to the tested conditions (Lüning 1981).

The F_v/F_m in *G. cornea* demonstrated a level of acclimation to low irradiance conditions (~100 µmol photons m^{-2} s^{-1}) employed for thallus maintenance in the laboratory. F_v/F_m is a widely used parameter for evaluating the maximum efficiency of photosystem II (PSII) and high values (Büchel and Wilhelm 1993; Maxwell and Johnson 2000) indicate

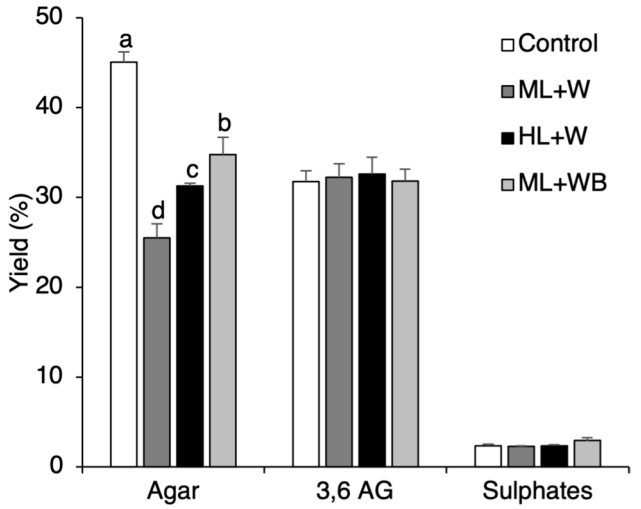

Fig. 4 Agar yield (%) in *Gracilaria cornea* along with their respective contents of 3,6-anhydrogalactose (3,6 AG) and sulfate (SO_4^{2-}) content in treatments exposed to 100 µmol photons m^{-2} s^{-1} (control); 350 µmol photons m^{-2} s^{-1} with white light (ML+W) and 700 µmol photons m^{-2} s^{-1} with white light (HL+W) during first week; 350 µmol photons m^{-2} s^{-1} with white and blue light (ML+WB) and 700 µmol photons m^{-2} s^{-1} with white and blue light (HL+WB) during the second week. HL+WB data is missing due to sample loss. Bars represent the mean ± standard deviation ($n = 3$). Significant differences among treatments are indicated by asterisks and different letters ($p < 0.05$)

acclimation over cultivation time, as described by Figueroa et al. (2009) for *Ulva lactuca* and *Sargassum vulgare*. The observed F_v/F_m values in *G. cornea* ranging from 0.5 to 0.6 remained stable and were similar to the range reported for red macroalgae in previous studies (Wedchaparn et al. 2015; Pliego-Cortés et al. 2019). However, weekly measurements of F_v/F_m during the experiment demonstrate relative acclimation to moderate-high irradiance in *G. cornea*. These F_v/F_m values are consistent with those reported by Álvarez-Gómez et al. (2019) for the same species.

The P_{max} values consistently decreased with cultivation time under high irradiance, whereas the opposite trend was observed under medium light intensity. This response suggests that *G. cornea* had limitations in coping with excessive light. It is plausible that the cumulative energy received during the second week of the high irradiance treatment resulted in photodamage. This is supported by the observation of damaged thalli at the end of the experiment and lower oxygen production when compared with post-illumination respiration data in HL+WB (Fig. 2a and b). This phenomenon aligns with the findings of Dawes et al. (1999), who documented a similar response in *G. cornea* from Florida coasts during summer periods, characterized by higher radiation levels compared to the rest of the year.

Furthermore, post-illumination respiration in *G. cornea* decreased during the first week in both treatments, but gradually increased under high irradiance over time, ultimately reaching a level similar to that of the control. We observed higher respiration rates in organisms exposed to high light intensity (800 µmol photons m^{-2} s^{-1}), leading to the conclusion that this increase is strongly associated with irradiance and events of high metabolic activity (Vásquez-Elizondo and Enríquez 2016).

The pigment profile in *G. cornea* decreased during acclimation at both light intensities. This response suggested that these irradiances were stressful for the organism during the first week, leading to a decrease in the pigment profile as a photoprotective mechanism. However, it was able to acclimate to medium light intensity in the second week when blue light was added, which reversed the pigment content to continue efficiently capturing light (Ramus 1981).

A positive correlation was observed between all the pigments evaluated and the P_{max} values. Notably, the increase in pigment concentration and P_{max} under combined white and blue light conditions may be attributed to the Emerson effect. The Emerson effect, first proposed by Emerson and Rabinowitch (1960) and further studied by Tollin (1962) and Zhen and van Iersel (2017), attributes a synergistic interaction between different wavelengths, particularly the interaction between short and long, leading to an enhanced effect on photosynthesis (Myers 1971).

The observed increase in total carotenoids in *G. cornea*, probably associated with violaxanthin synthesis, aligned with the same pattern as Chl *a*, suggests their role as electron captors and their contribution to optimizing light absorption processes (Morais et al. 2006). Moreover, the observed decrease in pigments at high irradiance during the second week aligns with the findings for gross photosynthesis. Carotenoids primarily function as photoprotectors in red algae, protecting the photosystems from irreversible damage. The presence of carotenoids is commonly associated with high irradiance conditions (Larkum and Barrett 1983). In our study, zeaxanthin and its precursor β-carotene were identified in *G. cornea*. Zeaxanthin was specifically identified in treatments exposed to high irradiance, assisting as a protective mechanism for PSI against excessive light (Xie et al. 2020; Fattore et al. 2021).

The increase in phycoerythrin (Pe) levels in *G. cornea*, especially under medium irradiance with the addition of blue light, aligns with the results of Zepeda et al. (2020). This increase was attributed to the stimulation of pigment synthesis, which is associated with enhanced light energy absorption and upregulation of genes related to pigment accumulation under blue light conditions (Zhang et al. 2022). The estimated pigment concentrations in *G. cornea* were similar to the results reported by Álvarez-Gómez et al. (2019) for the same species (synonym with *Hydropuntia cornea*). However, the Pe values in our experiment were lower than those reported in a previous study, where the thalli were exposed

to lower irradiances (~100 µmol photons m^{-2} s^{-1}), but with different light qualities. The Chl *a*:Pe ratio in *G. cornea* was lower in the control group, as expected, due to the low irradiance during the maintenance period. Similar effects were observed with the addition of blue light in ML+WB in the second week, indicating that light quality can influence phycoerythrin production under moderate irradiance.

The carbon and nitrogen content in algal tissues serves as an indicator of the overall health of the culture. Nitrogen limitation can disrupt various physiological and biochemical processes by introducing complexities into data interpretation. However, in this study, no significant differences were observed between the treatments for the percentages of carbon. Moreover, the nitrogen values in the tissues exceeded 2%, indicating that nitrogen was not a limiting factor in the experiment (Roleda and Hurd 2019).

Antioxidant system

The antioxidant capacity of the methanolic extracts of *G. cornea* exhibited a consistent trend across all three protocols. Overall, a decrease in the antioxidant activity of the thalli was observed in the treatment groups compared to that in the control group, which was exposed to low irradiance. Similar reductions in antioxidant capacity, as assessed by ABTS assay, were also reported by Schneider et al. (2022) in the same species. However, it is important to note that the thalli used in our study originated from outdoor cultures with fluctuating irradiance, suggesting that some of the antioxidant capacity may be attributed to the presence of residual metabolites (Pliego-Cortés et al. 2019). Polyphenols such as flavonoids are vital for the antioxidant defense mechanisms of macroalgae. They donate electrons to counteract reactive oxygen species. The production of phenols in *G. cornea* showed no significant differences among the treatments, which is consistent with previous studies involving different light colors and UV radiation exposure in this species (Schneider et al. 2022). Moreover, the detrimental effects of high irradiance on *G. cornea* may have been so severe that the synthesis of phenols became irrelevant.

The results of this study demonstrated a strong correlation between phenol production and antioxidant activity evaluated using the FRAP method in *G. cornea* extracts. Similar findings were reported by Pliego-Cortés et al. (2017), who observed a comparable relationship between total phenolic content and antioxidant activity assessed by the DPPH assay in *Rhodymenia pseudopalmata*. However, as emphasized by Álvarez-Gómez et al. (2019), the antioxidant activity in *H. cornea* is linked to thalli exposure to UV radiation, and the contribution of phenols to this activity should be negligible. Nevertheless, it has been established that these compounds could constitute a significant portion of the antioxidant capacity of methanolic extracts of seaweeds, particularly in red algae, where they stem from phenolic compounds, such as flavonoids and phlorotannins (Matanjun et al. 2008; Schneider et al. 2020).

We observed a decrease in the antioxidant capacity of phosphate buffer extracts, evaluated using FRAP and ABTS assays, during the first week for both irradiances. However, the addition of blue light increased the antioxidant capacity. A similar trend was observed in the synthesis of phycobiliproteins. In contrast, the antioxidant response evaluated by the DPPH assay showed a different tendency. Blue light led to a decrease in antioxidant activity at both irradiances. Overall, at moderate irradiance levels (~350 µmol photons m^{-2} s^{-1}), *G. cornea* displayed a stronger antioxidant response in phycobiliprotein extracts compared to methanolic extracts. Some studies have reported antioxidant activity in phycobiliprotein extracts from cyanobacteria (Sonani et al. 2014) and red macroalgae species like *Bangia atropurpurea* (Punampalam et al. 2018). Interestingly, the antioxidant capacity was higher in the phycobiliprotein extracts compared to the methanolic extracts. This is noteworthy because the use of phycobiliproteins as food colorants has been on the rise, and their antioxidant capacity could potentially enhance the value of derived products (Mysliwa-Kurdziel and Solymosi 2016; Pagels et al. 2019; Ali Anvar and Nowruzi 2021).

Polysaccharide synthesis

The synthesis of agar was significantly influenced by the level of irradiance. Moderate and high irradiance treatments resulted in a reduction in the agar yield compared to control thalli. This observation supports a previous study on *Gracilaria* sp. cultivated in the laboratory, where higher levels of agar were found under low irradiance conditions (Rotem et al. 1986). Freile-Pelegrín and Robledo (1997) reported the same effect in wild *G. cornea*, which observed higher agar content during the rainy season when irradiance is low, attributed to cloudy days along the Yucatán coast. Their results align with our findings in cultivated *G. cornea*. We also found similar agar content compared to that reported by Pereira-Pacheco et al. (2007) in wild organisms of the same species. Additionally, when we added blue light during the second week, the agar content increased under moderate irradiance. You and Barnett (2004) observed that exposure to blue light promotes increased polysaccharide production compared to white light. The sulfate content in *G. cornea* in our study was significantly low, as expected for agar, which is minimally sulfated (Hurd et al. 2014). Furthermore, *G. cornea* did not exhibit any changes in the 3,6 A-G, indicating that gel strength remained constant throughout the experiment. Our results highlight the potential for the commercial exploitation of agar from this *G. cornea* strain found in the Yucatán peninsula.

In conclusion, this study demonstrates that both irradiance and light quality significantly impacted the physiology, metabolite production, and antioxidant activity of *G. cornea*. Specifically, the combination of white and blue light stimulated the synthesis, quantity, and concentration of the evaluated variables in *G. cornea*. Nonetheless, it is worth emphasizing that various responses of the photosynthetic apparatus may be influenced by non-photosynthetic photoreceptors, like cryptochromes, which are responsive to blue light (Rüdiger and López-Figueroa 1992; Hegemann 2008). To comprehensively understand all responses of *G. cornea* to the combination of irradiance and light quality, it would be essential to identify the genes associated with the mechanisms that trigger these photoreceptors. This represents an interesting topic for future research. The data collected in this biological experiment shed light on the various strategies employed by *G. cornea* to adapt to the environmental conditions it was subjected to. Overall, given *G. cornea* limited tolerance to high light intensity, we observed that moderate irradiance consistently prevents thallus damage. Additionally, when combined with blue light, it promoted photosynthesis, pigment content and antioxidant capacity. These findings have applied implications and could be a valuable guide for optimizing the cultivation and utilization of *G. cornea* as a nutraceutical product in the pharmaceutical and cosmetic industries. Further studies and practical implementations can be pursued to capitalize on the findings and explore the feasibility of large-scale controlled cultivation of *G. cornea* for industrial purposes.

Supplementary information The online version contains supplementary material available at https://doi.org/10.1007/s10811-024-03317-8.

Acknowledgments The authors would like to thank C. Chávez for biochemical analysis. The first author is grateful to CONACYT Ph.D. scholarship N° 615458 and a research grant from the ISblue project, Interdisciplinary Graduate School for the Blue Planet (ANR-17-EURE-0015), co-funded by the French government under the program "Investissements d'Avenir' embedded in France 2030.

Authors' contributions EZ, RMV, YF and DR conceived and designed the study. EZ and RMV conducted the experiments. EZ, RMV and HP carried out laboratory work and data analysis. YF, NB and DR provided project administration and supervision. YF supported project funding. EZ drafted the manuscript. All authors edited and reviewed the manuscript and approved the final submitted version.

Funding Our research was funded by the NutraMar project of CONACyT (PN-CONACYT 2015-01-118).

Data availability The authors assert that the data underpinning the findings of this study are accessible within both the article and its supplementary information files.

Declarations

Competing interests The authors declare no competing interests.

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