



Growth and biochemical composition of the carrageenophyte *Eucheumatopsis isiformis* (Solieriaceae, Rhodophyta) under cultivation at different irradiance levels

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Abstract

Long-term production of a few species of *Eucheuma* and *Kappaphycus* from the Indo-Pacific Ocean supports the global carrageenan industry. The widespread use of vegetative propagation has resulted in low genetic diversity among farmed populations, making these crops increasingly vulnerable to diseases and environmental stressors, and resulting in declining yields and reduced biomass production over time. Insufficient efforts to develop and select new native cultivars, combined with the ecological risks posed by introducing nonindigenous species, underscore the urgent need to enhance cultivar diversification in eucheumatoid farming. *Eucheumatopsis isiformis*, a native carrageenophyte to the Atlantic Ocean, has potential for its cultivation in the Yucatán peninsula, Mexico. This study evaluated the growth and biochemical composition (pigments, elemental C and N, kappa/iota carrageenan ratio) of *E. isiformis* under different irradiance conditions in an indoor cultivation system. Specimens were collected in a subtidal population in Yucatán, Mexico, characterized, acclimatized to laboratory conditions, and exposed to five irradiance levels (71 ± 10.59 – $1014 \pm 65.85 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$; 23.4°C , salinity 33–34). Growth was measured weekly for six weeks, and elemental C and N content, pigment composition, and the kappa/iota-carrageenan ratio were evaluated. Growth and chromatic photoacclimation to light conditions were observed in all treatments. Highest growth rates (3.1 – $3.2\% \text{ day}^{-1}$) were registered between 238 ± 28.3 – $518 \pm 44.3 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$, with a predominant iota-type carrageenan (0.97 ± 0.02) observed in all treatments. This study aims to provide guidelines for the successful development of the aquaculture of this carrageenophyte.

Keywords Chromatic photoacclimation · Eucheumatoid · Rhodophyta · FTIR · Indoor cultivation

Introduction

Eucheumatoids comprises two of the most commercially important genera for the global carrageenan industry: *Eucheuma* J.Agardh and *Kappaphycus* Doty, whose species are mainly distributed in the Indian and Pacific Oceans (Hurtado et al. 2017). Around 98.8% of their

production by cultivation is focused on Asia (11,491,956 t wet weight), mainly in Indonesia and the Philippines, with some efforts also made in East Africa, Oceania, and Latin America and the Caribbean, which collectively represent less than 1.2% of the global production (FAO 2020, 2021). The main farmed eucheumatoids in Asia include a few species: *Eucheuma denticulatum*, *Kappaphycus alvarezii*, *Kappaphycus malesianus*, and *Kappaphycus striatus*. In 1970, the cultivation of eucheumatoids was successfully established in the Philippines. Since then, long-term production through vegetative propagation has resulted in low genetic variation, leading to poor seed quality, slow growth, high susceptibility to pathogens and diseases, and biomass loss, ultimately decreasing global production (Parenrengi et al. 2020; Dumilag et al. 2023). Insufficient efforts to select new native cultivars have led to a lack of diversification and the introduction of nonindigenous

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species into new territories, representing a risk for local ecosystems (Mallea et al. 2014; Castelar et al. 2015; Dumilag et al. 2023).

On the Atlantic coast of America, cultivation efforts for different eucheumatoids have been undertaken to achieve commercial scale, including tank cultivation, mariculture trials, experimental cultivation, and pilot-scale cultivation (Table 1). However, cultivation at a commercial scale has

been achieved only for the introduced species *K. alvarezii* on the Brazilian coast as a kappa-carrageenan producer (Reis et al. 2017). Different authors have suggested that the invasion risk of the introduced species *E. denticulatum*, *K. alvarezii*, and *K. striatus* depends on the environmental conditions of the area and the propagation strategy of the species (Bulboa et al. 2007; de Barros-Barreto et al. 2013; Castelar et al. 2015). In the southeast part of Mexico,

Table 1 Cultivation efforts of Eucheumatoids on the Atlantic coast of America

Currently accepted taxon	Distribution	Cultivation efforts
Introduced species		
<i>Eucheuma denticulatum</i> (N.L.Burman) Collins & Hervey	Caribbean Islands (Lesser Antilles) Tropical and Subtropical Western Atlantic	Cultivation trials in Cuba and ecological risk evaluation (Mallea et al. 2014; Hurtado et al. 2017) Cultivation trials in the Lesser Antilles (Guadeloupe) (Barbaroux et al. 1985) Cultivation trials (mariculture) in Venezuela show high growth rates (Smith and Renard 2002; Smith and Rincones 2006)
<i>Kappaphycus alvarezii</i> (Doty) L.M.Liao	Caribbean Islands (Cuba) Central America (Costa Rica) Tropical and Subtropical Western Atlantic	Mariculture trials in Yucatán, Mexico (Muñoz et al. 2004) Experimental mariculture and tank cultivation trials in Panama (Batista de Vega et al. 2004; Batista de Vega 2009) Experimental cultivation in Colombia (Rincones-León and Moreno-Tirado 2011) Cultivation trials (mariculture) in Venezuela with high growth rates (Smith and Rincones 2006) Experimental and commercial cultivation in Brazil with an annual production of 1200 t of wet weight (Castelar et al. 2009, 2015; Hurtado et al. 2017; Reis et al. 2017) Cultivation trials in Cuba, successful adaptation and evaluation of ecological risk (Mallea et al. 2014; Hurtado et al. 2017)
<i>Kappaphycus striatus</i> (F.Schmitz) L.M.Liao	Tropical and Subtropical Western Atlantic	Cultivation trials in Cuba, successful adaptation, and evaluation of ecological risk (Mallea et al. 2014; Hurtado et al. 2017) Pilot cultivation trials in Brazil using tetraspore progeny (Bulboa et al. 2007; de Barros-Barreto et al. 2013)
Native species		
<i>Eucheumatopsis isiformis</i> (C.Agardh) Núñez-Resendiz, Dreckmann & Senties previously known as: • <i>Eucheuma isiforme</i> (C.Agardh) J.Agardh (synonym) • <i>Eucheuma isiforme</i> var. <i>denudatum</i> D.P.Cheney (uncertain position taxa)	North America (Florida, Mexico, North Carolina) Western North Atlantic (Bermuda) Caribbean Islands (Bahamas, Barbados, Caribbean, Cuba, Guadeloupe, Hispaniola, Lesser Antilles, Puerto Rico, Virgin Islands) Central America (Belize, Costa Rica) Tropical and Subtropical Western Atlantic	Experimental cultivation (transplant) in the Florida Keys, USA (Dawes 1974) Experimental and pilot cultivation in Yucatán, Mexico (Perez-Enriquez 1996b, a; Robledo and Freile-Pelegrín 2011) Cultivation in Belize (Smith and Rincones 2006) Experimental cultivation in Colombia (Smith and Rincones 2006; Rincones-León and Moreno-Tirado 2011) Experimental cultivation in Venezuela (Montoya et al. 2020) Cultivation trials in the Lesser Antilles (Barbuda and St. Lucia) for local consumption (Smith and Renard 2002)

the native carrageenophyte *Eucheumatopsis isiformis* (C. Agardh) Núñez-Resendiz, Dreckmann and Senties, previously known as *Eucheuma isiforme*, has been reported as a potential source of a predominantly iota-carrageenan, with yield and viscosity levels similar to those required for industrial applications (Freile-Peigrín and Robledo 2006; Freile-Peigrín et al. 2006; Robledo and Freile-Peigrín 2011). This species has been harvested commercially in Antigua and Barbuda and Belize during the 1980s and 1990s, respectively. Harvested biomass was exported to the USA for health food applications or used in the region for traditional food applications; however, this practice ceased due to the overexploitation of the natural populations in the area (Smith and Renard 2002; Smith and Rincones 2006). In the Gulf of Mexico, *Eu. isiformis* was harvested from natural populations and exported to Denmark in the 1970s as a source of iota carrageenan, serving as an alternative to supply *E. denticulatum* from the Philippines (Freile-Peigrín et al. 2006). However, natural populations of the species in the Yucatán peninsula are not suitable for commercial harvesting or as a seedling supply due to their patchy distribution and the low biomass reported for the area, suggesting the need to develop its production through aquaculture (Perez-Enriquez 1996a, b; Robledo and Freile-Peigrín 2011).

Cultivation efforts of *Eu. isiformis* in the Atlantic coasts of America include experimental trials in the USA (Florida Keys), Colombia, and Venezuela; and pilot cultivation efforts in Cuba and the Lesser Antilles; reporting growth rates of 1–7% day⁻¹, which varies depending on the area, the season and the cultivation conditions (Dawes 1974; Smith and Rincones 2006; Rincones-León and Moreno-Tirado 2011; Montoya et al. 2020; Umanzor et al. 2020). In Florida, USA, it has been suggested that besides cultivation on the sea, a land-based nursery and tank-culture phase is needed to secure seed supply and provide reliable quantities of stock material, since loss of biomass could be a limitation in regions affected by severe storms during hurricane season (Umanzor et al. 2020). In the Yucatán coast at the Gulf of Mexico, mariculture efforts of eucheumatoids have been done demonstrating that environmental and oceanographic conditions in the region are suitable for tropical seaweed farming during the dry season and part of the wet season, which coincides with the closed season of the main fisheries, providing an alternative economic activity for fishermen in the area (Muñoz et al. 2004). However, as in the case of Florida, the Yucatán coast is affected by storms during the hurricane season, which caused the loss of biomass in the above-mentioned cultivation project that was interrupted by Hurricane Isidoro, which hit the Yucatán coasts in September 2002 (Robledo et al. 2013).

The cultivation of tropical red macroalgae is a financially sensitive endeavor, with profitability closely tied to algal growth rates. Although the variation in growth rates between minimum, average, and maximum levels is relatively small,

typically ranging from 3 to 4 percent per day, these differences can be critical. Depending on the cultivation technology employed, even slight reductions in growth can determine whether a production cycle results in a profit or loss (Robledo et al. 2025). Therefore, to develop commercially viable production of tropical macroalgae, particularly Eucheumatoids, it is essential to establish pilot projects and work with domesticated species that guarantee more stable growth rates.

Numerous studies conducted in both coastal and oceanic environments, under natural and cultivation conditions, have demonstrated that irradiance is the most critical and variable environmental factor influencing algal physiology and growth. It plays a key role in regulating the composition of photosynthetic pigments, the carbon and nitrogen content of algal tissue, and the yield and quality of carrageenan (Talarico and Maranzana 2000; Freile-Peigrín and Robledo 2006; Roleda and Hurd 2019). In particular, the heterogeneity in thallus coloration observed in *Eucheuma isiforme* from the sublittoral zone of coastal Florida, USA, has been linked to the varying light conditions under which the organisms develop (Dawes 1974; Mathieson and Dawes 1974). Pigment adjustments in response to photosynthetically active radiation (PAR) and ultraviolet (UV) exposure have also been documented in other tropical red algae. In these species, self-shading or extreme light exposure leads to reductions or increases in photosynthetic pigment concentrations, respectively, reflecting patterns of photoacclimation and underlying biochemical adaptations (Beach and Smith 1996; Godínez-Ortega et al. 2008). These acclimation responses support photoprotective mechanisms and optimize light capture, both of which are critical for coping with the physiological stresses typical of tropical environments. Consequently, a comprehensive understanding of how environmental factors influence species performance is essential for optimizing cultivation conditions, promoting growth while preserving the integrity of chemically valuable components.

This study aims to support the development of aquaculture and the domestication of the native carrageenophyte *Eu. isiformis* along the Yucatán coast by identifying suitable and viable strains. To achieve this, different color strains were collected and cultivated in an experimental indoor system to evaluate the effects of light on growth and chemical composition, including pigment content, elemental carbon and nitrogen levels, and the kappa/iota carrageenan ratio.

Materials and methods

Sampling of field-collected specimens and experimental design

Samples of *Eucheumatopsis isiformis* were collected between 2–3 m depth from a subtidal population in

Dzilam de Bravo, Yucatán, México (21°24'1.40"N, 88°54'35.90" W) in April 2023 and transported to the Applied Phycology Laboratory, Cinvestav Mérida. In the locality, the mean seawater temperature is 28.4 °C, ranging from 23 °C to 31 °C throughout the year, with a salinity between 29.6 and 35.6 (Freile-Pelegrín and Robledo 2006). The irradiance at the collection site ranged from 143–476 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$. Two distinct morphotypes of *Eu. isiformis* were identified among the field-collected specimens, based on key morphological and anatomical characteristics (Fig. 1a, b). It is worth noting that both morphotypes coexist in the same collection area and are exposed to similar environmental conditions. These are hereafter referred to as “smooth morphotype” and “spiny morphotype”, corresponding to the M1 and M3 morphotypes reported by Vázquez-Elizondo et al. (2025), respectively. The morpho-anatomical characteristics of both morphotypes coincided with previous descriptions of *Eu. isiformis*, previously reported as *Eucheuma isiforme* (Taylor 1960; Dawes et al. 1974; Cheney and Dawes 1980; Gabrielson 1983; Littler and Littler 2000; Núñez-Resendiz et al. 2019). Molecular marker analysis (*coxI*, *rbcL*, and RuBisCo spacer) confirmed the taxonomical identity of both morphotypes as *Eu. isiformis* (data under publication). The “smooth morphotype” exhibited greater susceptibility to contamination, often resulting in thallus degradation. In contrast, the “spiny morphotype”, characterized by spines arranged in whorls or a radial pattern, was more abundant and showed higher survival rates during laboratory acclimation. For that reason, as a starting point for establishing cultures under controlled conditions, specimens of the spiny morphotype from the subtidal population were used and characterized based on their perceived color using an RGB scale. The RGB color system constructs all the colors from the combination of the Red, Green, and Blue colors. The RGB (red, green, and blue) uses 8 bits each, which have integer values from 0 to 255. This makes $256 \times 256 \times 256 = 16,777,216$ possible colors. Additionally, their photosynthetic pigment and elemental composition were analyzed to develop an indicator relating color to biochemical composition. In the laboratory, one individual specimen of each color representing the range of pigmentation found in the natural population was separated, cleaned, and classified immediately after collection according to their predominant perceived color. Color characterization of the specimens was performed using digital photographs taken with an 18–55 mm lens mounted on a Canon Rebel T7 camera. All images were captured under consistent lighting conditions, with the camera fixed on a tripod and illuminated by a LED lamp. These photographs were analyzed using Just Color Picker 5.8 software to obtain color values

based on the RGB model. Subsequently, for each color variant, specimens were analyzed to determine dry weight (%), pigment content (chlorophyll *a*, phycoerythrin, and carotenoids), and tissue carbon and nitrogen content following the methods described in the next section. Subsamples from the same individuals ($n = 4$) were analyzed to ensure a representative assessment of the whole thallus composition.

Based on the biochemical content (chlorophyll *a* and phycoerythrin) which reflects the photosynthetic capacity, and the state of the thallus after acclimatization to laboratory conditions, the red-colored phenotype was selected for experimental cultivation as it showed higher survival and less susceptibility to contamination and degradation. After cleaning, clonal apical fragments of the red-color phenotype were acclimatized for 10 days under controlled conditions: light intensity of 60–80 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$, temperature of 23.4 °C, salinity of 33, a photoperiod of 12:12 h, weekly seawater exchange and nutrient pulses (Provasoli enrichment 1.28 mL L⁻¹, $\approx 82 \mu\text{M NO}_3^-$ and $8.2 \mu\text{M PO}_4^{3-}$) added once a week after water exchange. This nutrient pulse has been used for this species, as it prevented epiphyte growth and thallus degradation in preliminary trials. The experiment was conducted over six weeks in an indoor cultivation system to evaluate the effect of irradiance on the growth and chemical composition of the species. A total of 20 experimental units were used, each composed of an acrylic cylindrical vessel of 1 L capacity, filled with 800 mL of seawater and supplied with continuous aeration (Fig. 1c). Five irradiance levels were defined, using the saturating irradiance reported for this species as reference ($E_k = 270 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$; Vázquez-Elizondo et al. 2025): L1 ($\sim \frac{1}{2} E_k$) = $71.5 \pm 10.5 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$; L2 ($\sim 1 E_k$) = $238.2 \pm 28.3 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$; L3 ($\sim 2 E_k$) = $518 \pm 44.3 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$; L4 ($\sim 3 E_k$) = $801 \pm 73.6 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$ and L5 ($\sim 4 E_k$) = $1014.8 \pm 65.8 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$. LED lamps (Luma Light Studio Serie LA PRO-15065 Full White) were used as the lighting source. The L1 treatment ($71.5 \pm 10.5 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$), which was the lowest light level used, matched the irradiance level used during acclimation. Vessels were covered with 0, 1, 2, or 3 layers of black netting to achieve the desired light intensities for each treatment. Irradiance inside each vessel was measured using a quantum sensor (Spherical Micro Quantum Sensor US-SQS/L, Walz, Germany) connected to a data logger (ULM-500, Walz, Germany). Four experimental units ($n = 4$) were used for each light treatment. In each unit, two clonal apical fragments of approximately 1 g were used as the initial biomass, resulting in a mean initial density of $2.8 \pm 0.2 \text{ g L}^{-1}$. Cultivation

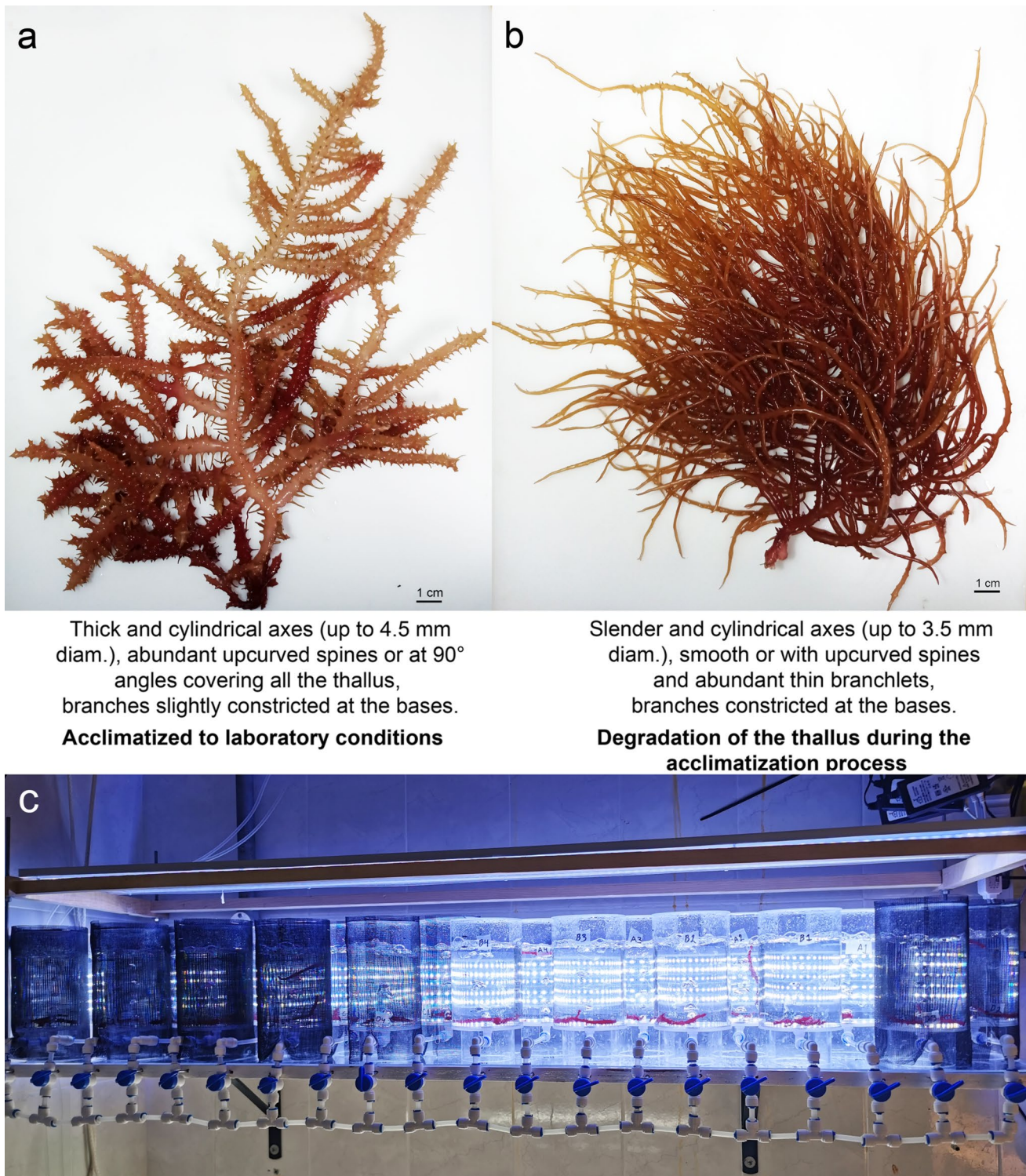


Fig. 1 *Eucheumatopsis isiformis* morphotypes from Yucatán, México. **a)** Spiny morphotype used for experimental trials; **b)** Smooth morphotype; **c)** Experimental setup of the cultivation system under five irradiance treatments (L1–L5)

conditions were maintained at 25.5 ± 0.6 °C, salinity of 33, a 12:12 photoperiod, weekly seawater exchange, and nutrient pulses as those described above for the acclimation period.

At the end of the six-week period, fragments were removed, and their color was characterized using digital photographs taken under the same conditions as those for the field-collected specimens. The images were analyzed

with Just Color Picker 5.8 software to obtain perceived color values based on the RGB model, focusing on pixels from the apical regions (< 1 cm from the apex).

Daily growth rate and biochemical analysis

Growth and density were monitored weekly throughout the six-week experiment by weighing the samples every seven days. Daily growth rates were estimated according to Yong et al. (2013) as $DGR (\% \text{ day}^{-1}) = [(W_t/W_0)^{1/t} - 1] \times 100$, where W_0 is the initial weight (g), W_t is the final weight (g) after the number of culture days t .

At the end of the experiment, photosynthetic pigments content chlorophyll *a* (Chl *a*), carotenoids (Car), and phycoerythrin (PE), dry weight, carbon (C) and nitrogen (N) content, and the ratio of kappa/iota carrageenan were determined for each treatment. A sequential extraction of fresh algae fragments for pigment analysis was performed in two steps in the dark. Fresh fragments of 0.7 g were ground with liquid nitrogen in a mortar. In the first step, 5 mL phosphate buffer (pH 6.8, 1 h) was added for phycobiliprotein extraction, centrifuged at 3000 rpm for 20 min (Sorvall ST 16R), and the supernatant was used for spectrophotometer readings at 565 nm. In the second step, the pellets were resuspended in 5 mL acetone (80% v/v) for the chlorophyll *a* and carotenoids extraction for 24 h at 4.1 °C. Subsequently, samples were centrifuged at 3000 rpm for 20 min, and the supernatant was used for spectrophotometer readings at 480 nm and 510 nm for carotenoids, and 663 nm for chlorophyll *a*. Pigments were calculated according to published equations (Dawes 1998): Chl *a* ($\text{mg g}^{-1} \text{ dw}$) = $11.9(A_{663}) \times [V/(wt) (\text{dw:fw ratio}) (1000)]$; PE ($\text{mg g}^{-1} \text{ dw}$) = $12.4(A_{565}) \times [V/(wt) (\text{dw:fw ratio}) (1000)]$ and Car ($\text{mg g}^{-1} \text{ dw}$) = $7.6 (A_{480} - (1.49(A_{510}))) \times [V/(wt) (\text{dw:fw ratio}) (1000)]$, where: *A* is the absorbance at specific wavelength indicated as subscript; *V* is the final volume of acetone or phosphate buffer in mL; *wt* is the fresh weight of the sample; and *dw:fw ratio* is the dry weight to fresh weight ratio.

For the dry weight content, 1 g of fresh samples ($n=4$) was weighed, dried in an oven at 60 °C for 24 h and weighed again to obtain the percentage of dry weight using an analytical balance (Precisa XT 220 A). The elemental C and N algal tissue content was analyzed on dry samples ground in a motorized mill (SpectraMill Ball Pestle Impact Grinder Model 1100, Chemplex Industries) for 3 min. Milled samples of 10 mg of dry weight were analyzed by combustion (950 °C) using a Flash 2000 Organic Elemental Analyzer with a Prepacked Quartz Reactor (Thermo Scientific, USA). The determination of gases was carried out using the Thermo Scientific EagerSmart Data Handling Software.

To determine kappa/iota carrageenan ratio, we used Fourier Transform Infrared Spectroscopy (FTIR) analysis. Qualitative characterization of carrageenan was done

directly on dried and ground algal material for each treatment. Milled dry samples ($n=4$) were measured using a Nicolet iS5 spectrophotometer (Thermo-Scientific, USA) by the attenuated total reflectance technique. The spectra were read 32 times per sample in a range of 4000–650 cm^{-1} to a spectral resolution of 4 cm^{-1} , and the results were reported as transmittance. The ratio between 800–801 and 845 cm^{-1} bands (kappa/iota ratio) in the FTIR spectra was evaluated and used as a qualitative parameter to determine kappa/iota hybridization (Freile-Pelegrín et al. 2006).

Statistical analysis

Friedman ANOVA for related samples was used to test daily growth rate significant differences among weeks for each treatment. Kruskal–Wallis Test was used to determine the effect of irradiance level on growth, photosynthetic pigment content (chlorophyll *a*, carotenoids, and phycoerythrin), and elemental composition (C and N). Subsequently, Bonferroni post-hoc comparisons were done to test significant differences between individual treatments. All statistical analyses were done using the IBM SPSS Statistics software (V25) and RStudio (2023.06.0).

Results

Characterization of color variants from a natural population

Classification of *Eu. isiformis* individuals collected from a natural population based on thallus coloration, using values derived from the RGB color scale, led to the identification of five distinct color variants: Yellow–brown, Red, Pink, Green, and Amber (Fig. 2a–d).

Analysis of photosynthetic pigment concentrations revealed significant differences among the color variants ($p < 0.05$). For chlorophyll *a* content, significant differences were found between color variants. The lowest chlorophyll *a* concentration was observed in the Amber individual ($55.4 \pm 7.8 \text{ mg g}^{-1} \text{ dw}$), while the highest concentration was found in the Red variant ($108.9 \pm 23.1 \text{ mg g}^{-1} \text{ dw}$), which was nearly twice that of the Amber specimen (Table 2). Phycoerythrin content also showed significant differences between color variants. The highest concentration was found in the Red specimen ($106.2 \pm 53.2 \text{ mg g}^{-1} \text{ dw}$), representing nearly four times the concentration observed in the Green specimen ($28.0 \pm 9.3 \text{ mg g}^{-1} \text{ dw}$). Similar differences were observed for the total carotenoid concentration between color variants. The highest concentration was found in the Green specimen ($29.2 \pm 6.8 \text{ mg g}^{-1} \text{ dw}$), representing nearly

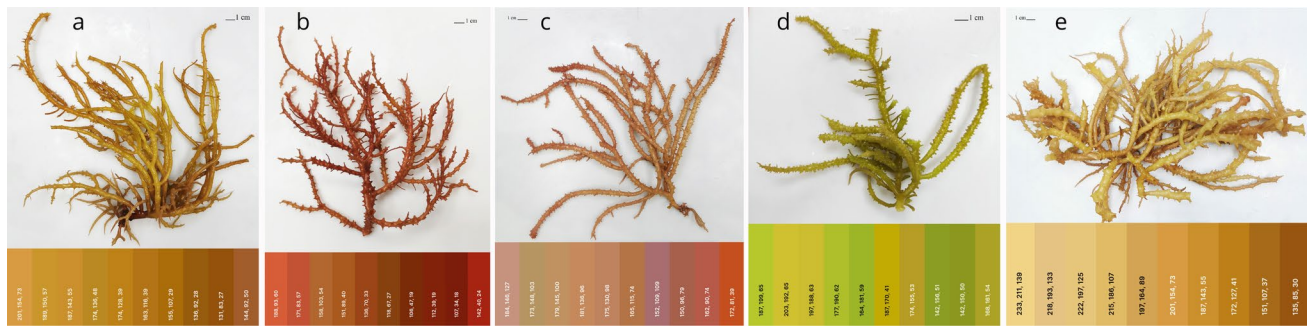


Fig. 2 Color characterization of *Eucheumatopsis isiformis* field-collected individuals. The RGB (Red, Green, Blue) color system, which has integer values from 0 to 255, makes $256 \times 256 \times 256 = 16,777,216$ possible color combinations. The RGB scale values shown at the bottom of each thallus refer to the predominant shades and colors present

in the thalli: **a)** Yellow–brown (174,136,48); **b)** Red (136,70,33); **c)** Pink (175,130,98); **d)** Green (164,181,59); **e)** Amber (197,164,89). The predominant perceived RGB color numbers are shown in brackets

Table 2 Biochemical analysis of *Eucheumatopsis isiformis* individuals from field collection in a subtidal population

Color morphotype	Dry weight (%)	Carbon (%)	Nitrogen (%)	C:N Ratio	Chlorophyll <i>a</i> (mg g ⁻¹ dw)	Phycoerythrin (mg g ⁻¹ dw)	Carotenoids (mg g ⁻¹ dw)
Green	15.34 ± 0.64 ^{a,b}	27.43 ± 0.89	0.82 ± 0.02	33.56 ± 0.73	94.20 ± 21.08 ^a	28.08 ± 9.34 ^a	29.20 ± 6.85 ^a
Red	16.54 ± 0.86 ^a	28.87 ± 2.5	0.8 ± 0.07	36.17 ± 0.8	108.94 ± 23.13 ^a	106.24 ± 53.22 ^b	20.07 ± 4.89 ^{a,c}
Pink	15.35 ± 0.33 ^{a,b}	29.75 ± 3.05	0.85 ± 0.31	38.09 ± 10.7	66.62 ± 17.79 ^{b,c}	77.38 ± 21.47 ^b	8.64 ± 1.98 ^b
Yellow–brown	13.77 ± 1.13 ^b	25.76 ± 3.62	0.7 ± 0.09	36.29 ± 2.58	86.83 ± 14.94 ^{a,b}	56.04 ± 17.24 ^{a,b}	21.00 ± 2.85 ^{a,c}
Amber	14.08 ± 0.85 ^b	25.98 ± 3.32	0.59 ± 0.04	41.47 ± 4.31	55.45 ± 7.84 ^c	40.53 ± 17.71 ^a	13.20 ± 2.74 ^{b,c}

Mean ± standard deviation (n = 4). For each component, different letters among color morphotypes indicate statistically significant differences

four times the lowest value observed in the Pink specimen (8.6 ± 1.9 mg g⁻¹ dw) (Table 2).

Dry weight content also showed significant differences between color variants. The highest dry weight percentage was observed in the Red specimen ($16.5 \pm 0.8\%$), while the lowest was found in the Yellow–brown specimen ($13.7 \pm 1.1\%$). The Green ($15.3 \pm 0.6\%$) and Pink ($15.3 \pm 0.3\%$) variants exhibited similar values (Table 2).

In contrast, elemental carbon (C) and nitrogen (N) content, as well as the C:N ratio, showed no significant differences between color variants. The highest C and N contents were observed in the Pink variant, with $29.7 \pm 3.0\%$ and $0.85 \pm 0.3\%$, respectively, while the lowest values were found in the Yellow–brown variant for carbon ($25.7 \pm 3.6\%$) and in Pink for nitrogen ($0.59 \pm 0.0\%$). Consequently, the highest C:N ratio was recorded in the Amber specimen (41.4 ± 4.3), and the lowest in the Green specimen (33.5 ± 0.7).

Growth under different irradiance levels

During the 10-day acclimation period, the daily growth rates of *Eucheumatopsis isiformis* ranged from 0–0.4%

day⁻¹. After that, the mean daily growth rates under different light treatments during the six-week cultivation period are shown in Fig. 3. All treatments exhibited positive growth throughout the experiment, with values exceeding 1% day⁻¹. However, the lowest mean growth rates were recorded during acclimation and in the L1 treatment, the last ranging from 0.5 ± 0.3 to $1.1 \pm 0.6\%$ day⁻¹. During the second week, a peak in growth was observed across all treatments except the L1, with the highest mean daily growth rates recorded in L2 and L3 ($3.2 \pm 0.7\%$ day⁻¹ and $3.1 \pm 0.7\%$ day⁻¹, respectively). A slight decrease in growth was noted in week three for treatments L3, L4, and L5, followed by a continuous decline from weeks four to six across all treatments. This downward trend coincided with an increase in biomass density, which reached 5.3 ± 0.6 to 7.0 ± 0.8 g L⁻¹ by the end of the experiment.

Biochemical analysis at the end of cultivation trials

The results showed that dry weight, carbon and nitrogen content, as well as phycoerythrin and carotenoid concentrations, differed significantly between irradiance levels at

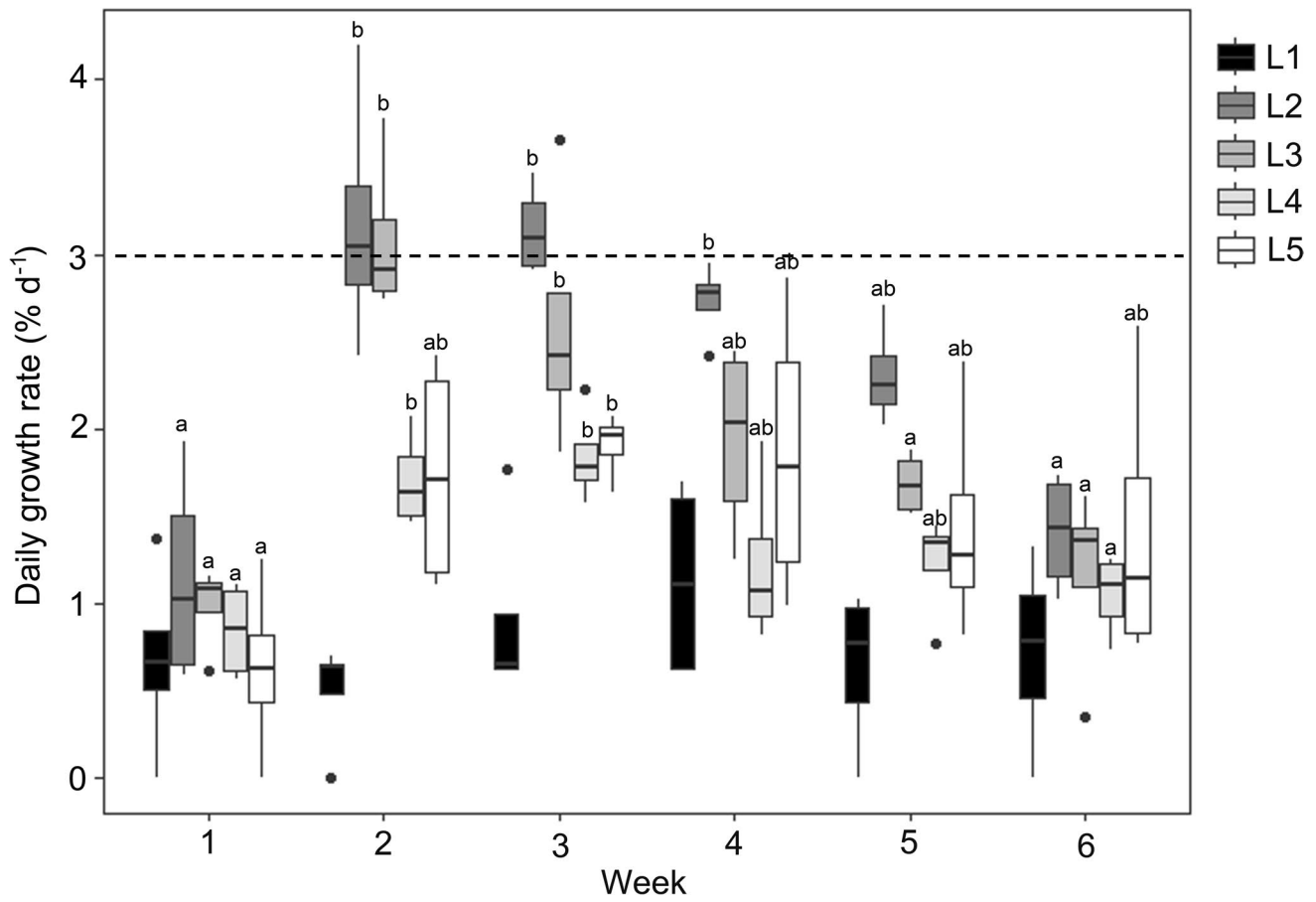


Fig. 3 Daily growth rates for *Eucheumatopsis isiformis* under different irradiance levels ($n=4$). **a)** $L1=71.5 \pm 10.5 \mu\text{mol quanta m}^{-2} \text{ s}^{-1}$; **b)** $L2=238.2 \pm 28.3 \mu\text{mol quanta m}^{-2} \text{ s}^{-1}$; **c)** $L3=518 \pm 44.3 \mu\text{mol quanta m}^{-2} \text{ s}^{-1}$; **d)** $L4=801 \pm 73.6 \mu\text{mol quanta m}^{-2} \text{ s}^{-1}$; **e)**

$L5=1014.8 \pm 65.8 \mu\text{mol quanta m}^{-2} \text{ s}^{-1}$. The dotted line indicates the minimum daily growth rate for commercial aquaculture. For each light treatment (L1–L5), different letters in the boxplots denote significant differences among weeks ($p < 0.05$)

the end of the experiment ($p < 0.05$) (Fig. 4). The lowest mean dry weight ($10.2 \pm 0.4\%$), carbon ($18.2 \pm 0.9\%$), and nitrogen ($2.3 \pm 0.1\%$) contents were observed under an irradiance of $238.2 \pm 28.3 \mu\text{mol quanta m}^{-2} \text{ s}^{-1}$ (L2, E_k), which also registered the highest mean growth rate ($3.2 \pm 0.7\% \text{ day}^{-1}$). In contrast, the highest mean dry weight ($14.2 \pm 1.2\% - 14.3 \pm 0.9\%$), carbon ($23.1 \pm 0.5\% - 23.0 \pm 0.6\%$), and nitrogen ($3.0 \pm 0.1\% - 3.0 \pm 0.2\%$) contents were found at the highest irradiances levels between $801 \pm 73.6 \mu\text{mol quanta m}^{-2} \text{ s}^{-1}$ (L4, $3E_k$) and $1014.8 \pm 65.8 \mu\text{mol quanta m}^{-2} \text{ s}^{-1}$ (L5, $4E_k$), where mean growth rates were comparatively lower, ranging from $0.6 \pm 0.5\% \text{ day}^{-1}$ and $1.9 \pm 0.2\% \text{ day}^{-1}$.

Regarding pigments, phycoerythrin content was highest under the lowest irradiance level of $71.5 \pm 10.5 \mu\text{mol quanta m}^{-2} \text{ s}^{-1}$ (L1: $\frac{1}{2} E_k$), reaching $342.3 \pm 94.3 \text{ mg g}^{-1} \text{ dw}$. Its concentration decreased with increasing irradiance, with the lowest values observed at the highest light levels, $176.7 \pm 22.6 \text{ mg g}^{-1} \text{ dw}$ and $186.6 \pm 29.1 \text{ mg g}^{-1} \text{ dw}$ under $801 \pm 73.6 \mu\text{mol quanta m}^{-2} \text{ s}^{-1}$ (L4: $3E_k$) and $1014.8 \pm 65.8 \mu\text{mol quanta m}^{-2} \text{ s}^{-1}$ (L5: $4E_k$), respectively.

Carotenoids exhibited an inverse pattern to phycoerythrin, with the highest content observed at the highest irradiance level ($31.1 \pm 6.6 \text{ mg g}^{-1} \text{ dw}$ at $1014.8 \pm 65.8 \mu\text{mol quanta m}^{-2} \text{ s}^{-1}$) and the lowest at the lowest irradiance ($14.6 \pm 4.2 \text{ mg g}^{-1} \text{ dw}$ at $71.5 \pm 10.5 \mu\text{mol quanta m}^{-2} \text{ s}^{-1}$). In contrast, chlorophyll *a* content showed no significant differences among treatments (Fig. 4).

Color characterization of *Eu. isiformis* fragments exposed to different irradiance levels in the controlled laboratory culture system was conducted using RGB color analysis. The resulting color palette revealed a clear gradient across treatments, with the darkest pigmentation observed under the lowest irradiance ($71.5 \pm 10.5 \mu\text{mol quanta m}^{-2} \text{ s}^{-1}$) and the lightest under the highest irradiance ($1014.8 \pm 65.8 \mu\text{mol quanta m}^{-2} \text{ s}^{-1}$) (Fig. 5).

Carrageenan characterization through FTIR

Fourier Transform Infrared Spectroscopy (FTIR) analysis revealed spectra consistent with the preservation of

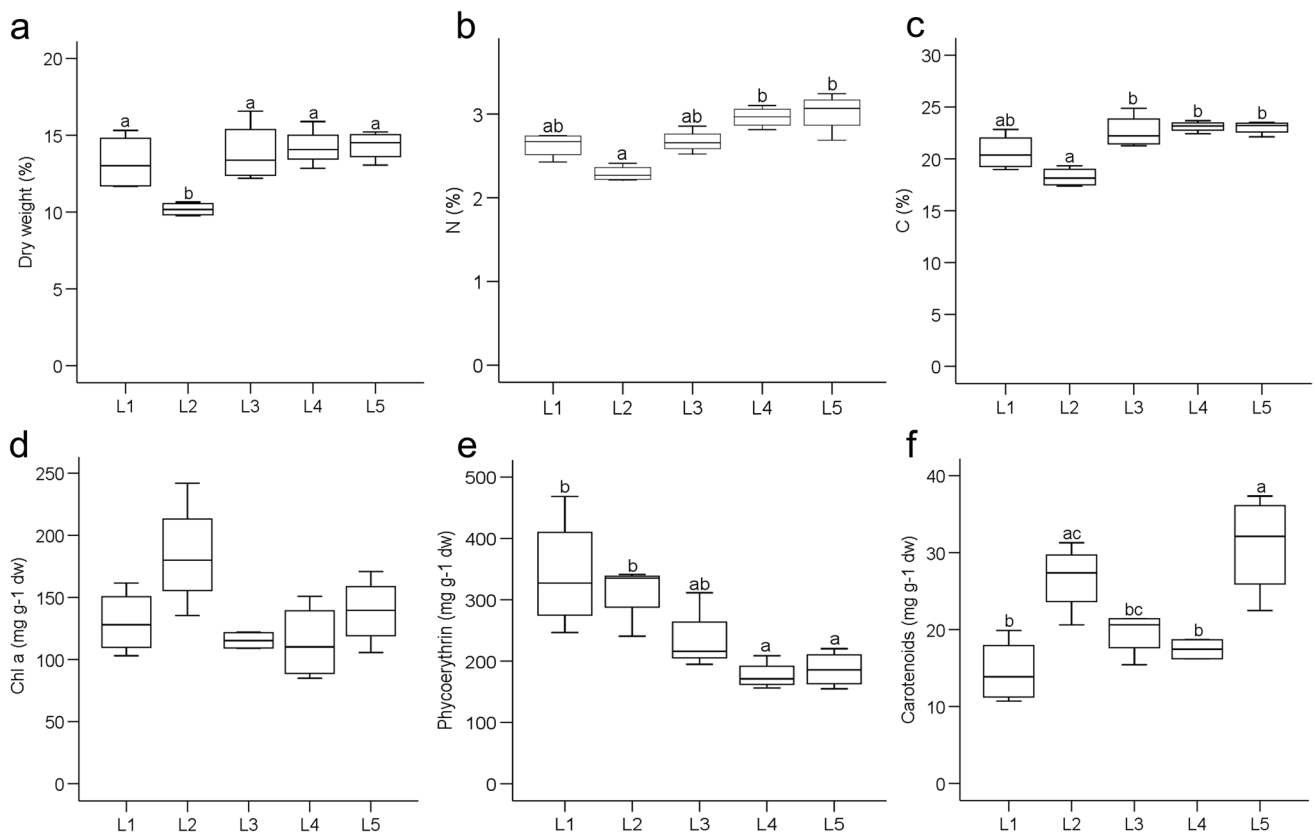


Fig. 4 Biochemical analysis of *Eucheumatopsis isiformis* exposed to five irradiance treatments ($n=4$); L1= $71.5 \pm 10.5 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$; L2= $238.2 \pm 28.3 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$; L3= $518 \pm 44.3 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$; L4= $801 \pm 73.6 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$;

L5= $1014.8 \pm 65.8 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$. **a)** Dry weight; **b)** Nitrogen; **c)** Carbon; **d)** Chlorophyll *a*; **e)** Phycoerythrin, and **f)** Carotenoid content. Different letters in the boxplots denote significant differences among light treatments ($p < 0.05$)

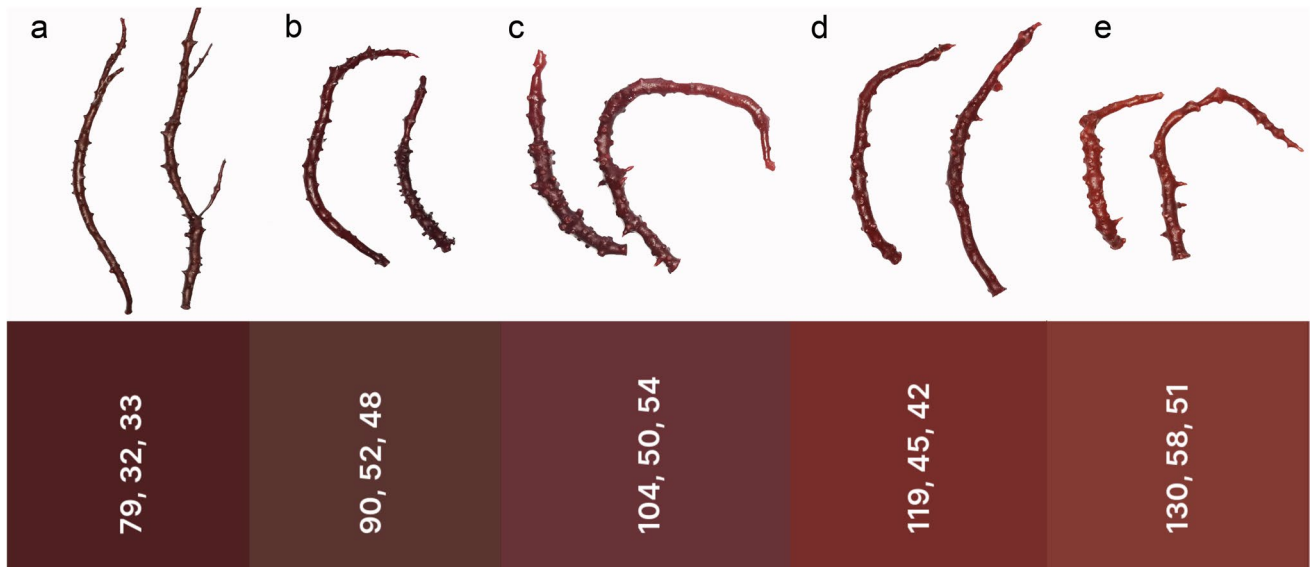


Fig. 5 Color characterization of *Eucheumatopsis isiformis* thalli subjected to five irradiance levels at the end of the experiment under controlled culture conditions ($n=4$). **a)** L1= $71.5 \pm 10.5 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$; **b)** L2= $238.2 \pm 28.3 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$; **c)** L3= $518 \pm 44.3 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$; **d)** L4= $801 \pm 73.6 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$;

e) L5= $1014.8 \pm 65.8 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$. The RGB (Red, Green, Blue) color system, which has integer values from 0 to 255, makes $256 \cdot 256 \cdot 256 = 16,777,216$ possible color combinations. RGB values were included at the bottom of each thallus to indicate the predominant color in each condition

the carrageenan structure across all irradiance treatments (Fig. 6). All spectra displayed an absorption bands at 1220 cm^{-1} related to sulfation level; 965 cm^{-1} associated to the presence of 3-linked β -D-galactose 4-sulfate and 4-linked α -D-galactose 2-sulfate; 928 cm^{-1} linked to the presence of 3,6 AG; 867 cm^{-1} related to the sulfate group in C-6 of nu-carrageenan; 845 cm^{-1} associated to 1,3 galactose-4-sulfate; and 803 cm^{-1} associated to 3,6-anhydrogalactose-2-sulfate. Qualitative characterization indicated the presence of a hybrid iota–kappa carrageenan. The mean ratio between the absorption bands at $800\text{--}801\text{ cm}^{-1}$ and 845 cm^{-1} (kappa/iota ratio) suggested a predominance of the iota type (0.97 ± 0.02). Among treatments, L3 showed the lowest ratio (0.95), while L2

exhibited the highest ratio (1.00), corresponding to a pure iota-carrageenan profile.

Discussion

Wild eucheumatoids are typically phenotypically plastic, exhibiting diverse morphologies and a wide range of colors (Roleda et al. 2021; Gelli et al. 2024). The experimental responses observed under different light treatments in this study are consistent with the environmental conditions where *Eu. isiformis* naturally occurs. The color variants characterized from field-collected specimens align with the descriptions provided by Dawes (1974) and Mathieson and

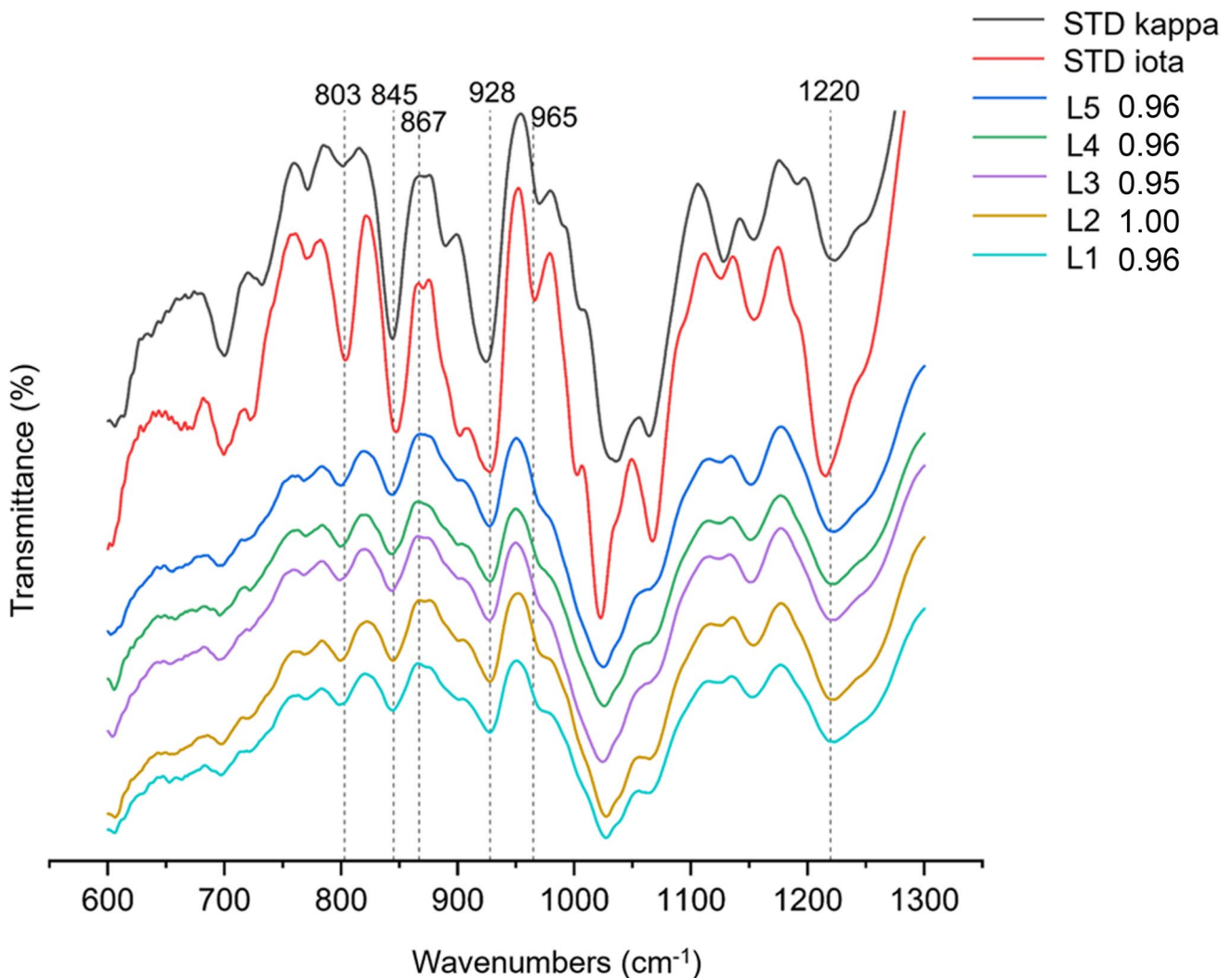


Fig. 6 FTIR spectra for the carrageenan of *Eucheumatopsis isiformis* under five irradiance levels (L1–L5). Lines indicate the relevant wavenumbers for carrageenan as described in the results section. The

kappa/iota ratio is indicated next to each treatment. Spectra of κ - and ι -commercial carrageenan standards included (STD)

Dawes (1974), who reported heterogeneous thallus coloration in *Eu. isiformis* and linked pigment variation to ambient light conditions along the sublittoral coast of Florida, USA. According to these authors, yellow, brown, and light green thalli are typically found in areas exposed to sunlight or at apical zones of the thallus, while reddish and dark brown coloration is commonly associated with basal regions or individuals growing in shaded environments. These observations coincide with our findings, where *Eu. isiformis* exhibited a range of colors and tonalities probably linked to the environmental heterogeneity of the collection site. The area included sandy zones fully exposed to light, patches covered by seagrass, and benthic vegetation overlaid by red algal mats, all of which contribute to the formation of diverse microenvironments. Thus, the constructed color palettes effectively captured the heterogeneity of coloration observed among the characterized organisms (Fig. 2).

In *Eu. isiformis*, reddish colors are expressed when phycoerythrin content is higher, whereas yellowish-green thalli have higher chlorophyll *a* and carotenoids. As for the pale-colored thalli, these had low concentrations of chlorophyll *a*, phycoerythrin, and carotenoids, in addition to the highest C:N ratio, which indicates low nitrogen content. This has been reported in *Gracilariopsis gracilis*, indicating photoinhibition and nitrogen deficit (Robertson-Andersson et al. 2009). Such information is important to consider if one seeks to select individuals with higher photosynthetic pigment reserves. The results in *G. gracilis* agree with Stadnichuk and Tropin (2017), who stated that photopigments and, especially phycoerythrin, function as nitrogen reserve sinks in red algae. However, in the present study, no clear relationship was found between phycoerythrin content and nitrogen levels among the *Eu. isiformis* color variants (Table 2). Therefore, it is not possible to infer the nitrogen content from the RGB-based color scale of the thallus in *Eu. isiformis*, as has been reported for other species. This limitation is likely due to the heterogeneity in pigmentation caused by chromatic adaptation to varying light conditions, as well as the presence of other nitrogen-containing compounds, such as nucleic acids, amino acids, proteins, and inorganic nitrogenous substances (Harrison and Hurd 2001). Unlike *Ulva fenestrata*, where a color scale successfully relates color intensity to nitrogen content (Stedt et al. 2022), the presence of phycobiliproteins and other compounds in *Eu. isiformis* complicates establishing a direct relationship between thallus coloration and nitrogen concentration. Therefore, using RGB color as an indicator for the nutritional status of the thallus, particularly nitrogen content, is not applicable in this species.

Experimental cultivation of the spiny morphotype of *Eu. isiformis* under varying irradiance levels showed positive growth rates across all treatments, ranging from 0.5 ± 0.3 to $3.2 \pm 0.7\%$ day⁻¹, indicating a broad tolerance

to high irradiance, up to $1014.8 \pm 65.8 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$. According to Munawan and Ruslaini (2021), a commercially acceptable daily growth rate for *Eucheuma* species is over $3\% \text{ day}^{-1}$. The highest growth rates (up to $3.2 \pm 0.7\% \text{ day}^{-1}$) were found under $238.2 \pm 28.3 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$, a value close to the saturation irradiance reported by Vázquez-Elizondo et al. (2025) for the species under laboratory conditions. Even with a twofold increase in saturation irradiance ($2E_k$: $518 \pm 44.3 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$), a commercially acceptable growth rate up to $3.1 \pm 0.7\% \text{ day}^{-1}$ was achieved in this study, which is higher than reported previously in a similar experimental culture system for the same species ($1.5\text{--}1.8\% \text{ day}^{-1}$) (Vázquez-Elizondo et al. 2025). Moreover, under saturation irradiance (E_k), the lowest dry weight and carbon content were registered. According to Freile-Pelegrín and Robledo (2006), an inverse relationship between dry weight and daily growth rate could indicate efficient growth periods of the species, reducing the carrageenan synthesis and its accumulation in the cell wall. Although daily growth rates in the other treatments ($\frac{1}{2}E_k$, $3E_k$, and $4E_k$) were lower than commercially acceptable levels, all fragments survived until the end of the experiment and exhibited positive growth across all irradiance treatments. In *Eucheuma denticulatum*, a broad range of positive growth rates has been reported (0.1 to $7.1\% \text{ day}^{-1}$) when exposed to different nutrient sources and treatments ($\text{NH}_4^+:\text{NO}_3^-$ ratio) under the same irradiance level ($178 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$), with no differences among nutrient treatments (Narvarte and Roleda 2025). This suggests that eucheumatoids are highly tolerant not only to different irradiance levels but also to the inorganic nitrogen source and level by adjusting the nitrogen utilization strategy (Narvarte and Roleda 2025). However, this response to nutrients has been demonstrated to be species-specific and strain-specific for different eucheumatoids (Narvarte et al. 2022, 2024, 2025). Further experimental studies are required to explore the interactions between environmental parameters such as light, nutrients, and temperature and their effects on the different morphotypes of *Eu. isiformis*, as these play a crucial role in the physiological performance of seaweeds.

Regarding growth patterns throughout time, no significant differences were observed between irradiance treatments during the first week (ranging from 0.6 ± 0.5 to $1.1 \pm 0.6\% \text{ day}^{-1}$), likely due to an initial acclimatization period. Barsanti and Gualtieri (2023) have reported that algae species face light variations through the photoacclimation process, which starts from the first hours and concludes after a few days. Vázquez-Elizondo et al. (2025) reported that *Eu. isiformis* takes one or two weeks to adapt the photosynthetic system and achieve the optimum function in the new light conditions. At the end of the second week, a peak in growth was observed in L2 to L5 treatments and maintained until the end of the third week, after which daily growth rates decreased. For the L1 treatment, no differences in growth

were found throughout the time, with growth rates 0.5 ± 0.3 to $1.1 \pm 0.6\%$ day⁻¹. The decrease in growth observed in L2 to L5 from the fourth week onwards was probably related to the increase in the biomass density, which could have increased the nutrient demand inside the cylinders. As stated by Barsanti and Gualtieri (2023), a limitation process occurs as density increases, limiting resources as light and nutrients, and modifying water parameters (pH and CO₂). The growth limitation induced by density increase in algae culture has been reported previously for other red seaweeds (Ullah et al. 2023).

The significant differences in accessory photosynthetic pigment content between irradiance treatments (Fig. 4) indicate photoacclimation and chromatic adaptation processes in response to changes in irradiance during experimental cultivation, as evidenced by the variation in thallus color (Fig. 5). Dawes (1974) and Mathieson and Dawes (1974) reported the reversible character of the color of the thallus of *Eu. isiformis* when grown under changing light conditions. Phycobiliproteins (e.g., phycoerythrin), in addition to their function in light harvesting, also act as nitrogen reservoirs. Both accessory pigments and chlorophyll *a* influence thallus color; however, they exhibit different responses to environmental conditions. For example, our results from laboratory cultures showed that carotenoids increased in response to high light intensities, since they have a photoprotective function, whereas phycoerythrin decreased in excess light, and chlorophyll *a* remained constant (Fig. 3). Furthermore, in high light conditions, despite a low concentration of phycoerythrin was found with a slightly lighter thallus color than in low light, the highest N content was recorded. These complicate the establishment of a linear relationship between RGB color and nitrogen content. Therefore, unlike what has been found in green algae, there are more variables to consider and relate the visual coloration of the thallus to the nitrogen content as a color scale. Initial nutritional status of the algae may have influenced nitrogen accumulation during preliminary culture, as final nitrogen values were higher in color variants with higher initial nitrogen and photosynthetic pigment concentration. This agrees with Roleda and Hurd (2019), who mention that the biological stage and nutritional status of the alga influence metabolic processes and the adaptive response of the organism.

There was a significant difference in phycoerythrin content between L1 ($\frac{1}{2} E_k$) and L5 ($4 E_k$) treatments, with the phycoerythrin content increasing while irradiance decreased, in coincidence with previous reports for *Eu. isiformis* (Moon and Dawes 1976). According to Stadnichuk and Tropin (2017), phycoerythrin increases the usable light spectrum by integrating 450 and 560 nm, which is an abundant spectrum range in depth and shaded habitats. Otherwise, results showed that *Eu. isiformis* decreased the phycobiliprotein content while increasing carotenoid content under high

irradiances. Carotenoids play a crucial role in protecting seaweeds against damage under excessive light conditions by acting as antioxidants and scavenging reactive oxygen species (Pangestuti et al. 2018; Cikoš et al. 2022).

Changes in tropical red algal pigmentation appear to take place within individual thalli as a result of photoacclimation (Beach and Smith 1996). This was evident in *Eu. isiformis* pigment content over the experimental treatments. This is a common observation in algae found in the sublittoral zone, where light is naturally limited and the algae themselves contribute to self-shading. Light treatments influenced the growth and pigment content of *Eu. isiformis* in culture and can be applied to manipulate also biochemical composition (Godínez-Ortega et al. 2008; Zepeda et al. 2025), suggesting the importance of strains with desirable characteristics for cultivation and the effect of environmental factors on these characteristics.

In the present study, carrageenan yields were not determined due to the low biomass availability at the end of the experiment, which was insufficient to perform extractions. However, the qualitative characterization of the carrageenan through FTIR analysis was done directly on dried and ground algal material, as done previously for other brown and red seaweeds, including *E. denticulatum* (Chopin and Whalen 1993; Gómez-Ordóñez and Rupérez 2011). The results showed a kappa-iota hybrid carrageenan, predominantly iota type, with an ι/κ ratio ranging from 0.95 to 1.00 (pure iota-carrageenan) under the irradiance treatments, whose values are in the range of those reported for other carrageenophytes used industrially (0.59–1) (Pereira et al. 2009). Previous evaluation of the ι/κ ratio for *Eu. isiformis* from Yucatán, Mexico, and Nicaragua have been done in the extracted carrageenan, with values ranging from 0.63 to 1.00 depending on the extraction conditions (native vs alkali-treated and extraction time) (Freile-Pelegrín et al. 2006; Freile-Pelegrín and Robledo 2008). Variations in the ι/κ ratio in different carrageenophytes have been related to the duration of cultivation and the amount of precursor (*mu*) at the time of harvest, the irradiance and nutrient availability, and tissue age (Fournet et al. 1999; Mendoza et al. 2006; Pereira et al. 2009; Villanueva et al. 2009). In a parallel study performed with *Eu. isiformis* from the same locality, it was observed that after 6 weeks under indoor cultivation conditions (27.8 ± 0.5 °C, salinity 35.8 ± 0.64), the native carrageenan yield decreased from $45.3 \pm 2.5\%$ in low light conditions (24.7 ± 9.4 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$) to $36.3 \pm 2.8\%$ in high light conditions (575.7 ± 32.1 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$) as well as the alkali treated carrageenan, decreasing from $42.1 \pm 1.1\%$ to $34.4 \pm 2.2\%$, respectively (Vázquez-Eli-zondo et al. 2025). This agrees with the inverse relationship between carrageenan yield and incident light reported by Freile-Pelegrín and Robledo (2006) for natural populations of *Eu. isiformis* from the Yucatán coast. Their study, which

evaluated seasonal variation, found carrageenan yields ranging from $33.2 \pm 1.6\%$ to $45.5 \pm 0.4\%$, with the highest yields occurring during the late rainy to early cold seasons, periods associated with lower incident light. In other carrageenophytes, it has been suggested that at high irradiances, the increase in photosynthesis promotes floridean starch accumulation, thus decreasing the synthesis of carrageenan. Conversely, at low irradiances, when photosynthesis is limited, carbon is transferred from stored floridean starch for use in carrageenan biosynthesis (Fournet et al. 1999).

Conclusions

Eucheumatopsis isiformis, a species native to the Americas, has been considered a potential alternative source of carrageenan to the species commonly used in industrial applications. However, its patchy distribution and the low biomass values reported along the Yucatán coast have driven efforts to develop cultivation techniques in the region. The results presented here provide valuable insights into the aquaculture potential of *Eu. isiformis* (spiny morphotype) from Yucatan. The cultivar demonstrated high tolerance to elevated irradiance levels, beyond saturation irradiance, likely due to chromatic photoacclimation, and achieved suitable daily growth rates for commercial cultivation at irradiance levels between 238 and $518 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$. The hybrid carrageenan extracted exhibited a consistently high iota-carrageenan content, with a mean ratio of 0.97 ± 0.02 and minimal variation across treatments (0.95–1.00), indicating a stable hybrid structure. As stated above, achieving adequate daily growth rates without nutrient supplementation is desirable for commercial cultivation, since increased nutrient availability may negatively impact the content and quality of phycocolloids. Nonetheless, further research is necessary to assess carrageenan biosynthesis under varying cultivation conditions to optimize growth without compromising carrageenan yield or quality.

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Vázquez-Delfín, Adrian Fagundo-Mollienda, Yolanda Freile-Pelegrín; Project administration: Daniel Robledo; Resources: Daniel Robledo, Yolanda Freile-Pelegrín; Supervision: Erika Vázquez-Delfín, Daniel Robledo; Validation: Erika Vázquez-Delfín, Daniel Robledo, Adrian Fagundo-Mollineda, Yolanda Freile-Pelegrín; Visualization: Roberto Villagrán-Torres; Writing – original draft: Erika Vázquez-Delfín, Roberto Villagrán-Torres; Writing – review & editing: Daniel Robledo.

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Data availability Data can be provided upon request.

Declarations

Competing interests The authors declare no competing interests.

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