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RESEARCH

The Effect of High Temperatures on the Growth and Biochemical Composition of Cultivated *Palmaria Palmata* (Rhodophyta)

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Abstract

Macroalgae are emerging as a promising resource for multiple applications in food and pharmaceutical industries owing to their potential as a rich resource of both nutritional and bioactive compounds. Here, we explore the influence of environmental conditions on the biochemical composition of the red seaweed *Palmaria palmata*, in relation to their growth potential. *Palmaria palmata* specimens were grown under different conditions for twelve weeks, including temperatures of 12, 15, and 17 °C, irradiance levels of 14 and 19 µmol m^{-2} s^{-1} and two nutrient conditions. Growth was assessed by measuring the relative growth rates of the seaweed. Biochemical composition (i.e., sugar, lipid, fatty acid, phycobiliprotein, and nitrogen content) was evaluated with Fourier-transform infrared spectroscopy for qualitative data and spectrophotometric and gas chromatography-mass spectrometry for quantitative analysis. The polyunsaturated fatty acid content of *P. palmata* peaked under nutrient-rich conditions at 12 °C and 14 µmol m^{-2} s^{-1}, which coincided with the highest observed growth rate of the seaweed. In contrast, higher temperatures exhibited a positive correlation with protein and xylan content, although this was accompanied by a decrease in antioxidant properties. Besides, galactose-rich compounds and R-phycoerythrin content were significantly higher in *P. palmata* grown at 12 °C. The study effectively showed that the growth and chemical composition of *P. palmata* vary under different environmental conditions, demonstrating potential to be a source of different nutritious and health-promoting compounds by modulating the culture conditions that can maximize specific compounds able to provide health benefits.

Keywords Nutritional value; fatty acids · Carbohydrates · Bioactive compounds · Pigments · Antioxidant activity

Introduction

Seaweed aquaculture is a growing industry worldwide, reaching a record of 35.1 million tonnes (fresh weight) of algae for both food and non-food uses, worth 16.5 billion US$ (FAO 2022). Seaweed cultivation has increased throughout the years around the world particularly in Asia (Naylor et al. 2021). The produced biomass has interesting functional and nutritional properties and can be used directly as food or feed (Torres et al. 2019), or turned into value-added products such as functional foods, cosmetics, nutraceuticals, or pharmaceuticals (Hafting et al. 2015). For centuries, seaweed has been part of the daily diet of some countries, particularly in East Asia, but they are still underexplored as food in Western countries. Currently, Europe is importing nearly the complete demand for seaweed from Asia, with China, Indonesia and the Republic of Korea being the major exporters (FAO 2022). The main uses of seaweeds in the

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Western world are mostly restricted to seaweed extracts for producing hydrogels, despite a living tradition of eating whole seaweeds in coastal areas, e.g., in Brittany (France), Norway, Wales, Scotland, Ireland, and Iceland (Mouritsen et al. 2013). The red macroalga *P. palmata*, commercially known as dulse, has a rich cultural history, as it has been eaten for centuries by people living along coastlines in the northern Atlantic, where the species is commonly found within a latitude range of approximately 40 to 80 °N (Mouritsen et al. 2013; Stévant et al. 2023). Here, the species is most found within the first 10 m below the surface in the intertidal and subtidal zones of coastal areas, but it can be observed at depths reaching up to 20 m in offshore shoals with exceptionally clear waters (Stévant et al. 2023). Light is a major factor limiting its vertical distribution and light saturation point estimates were observed to range from 75 to 400 µmol photon m^{-2} s^{-1} for *P. palmata* (Kübler and Raven 1995; Martínez and Rico 2008). The relatively high bulk retail value (business-to-business) of dried dulse has doubled/tripled compared to approximately a decade ago and is currently estimated between 40 and 75 € kg^{-1} by Stévant et al. (2023), while a decade ago, in 2011, price per kg bagged dry weight was assumed to be ranging between 21 and 26 € kg^{-1}, equivalent to 3.7 € kg^{-1} wet weight (inflation corrected numbers; Werner and Dring 2011).

A significant variation in the biochemical composition of many seaweed species has been described in literature (e.g., Holdt and Kraan 2011; Stévant et al. 2023), attributed to differences in environmental growth conditions such as temperature, irradiance, salinity, and nutrient composition of the water, possibly in combination with genetic differences between populations. Among these metabolites, photosynthetic pigments have attracted great attention from the scientific community thanks to their spectral behaviour, fluorescence, and colorant properties. Like for other compounds, most studies have focused on developing or refining extraction protocols for these pigments or in the study of seasonal fluctuations in their levels (e.g., Aguilera et al. 2002; Martins et al. 2021; Manzoor et al. 2024; Pereira et al. 2012), which may not align with the optimal production of these compounds within the macroalgae. The varying responses to environmental factors lead to one of many challenges in seaweed cultivation, which is, the optimal cultivation conditions for biomass yield may not correspond to the optimal conditions for the desired chemical composition.

Seaweed is known to respond to low nutrient availability by redistributing nutrient allocation to essential processes, such as cell maintenance and reproduction, at the expense of growth (Jevne et al. 2020). Limited research is available that aims to better understand how growth and biochemical composition might be influenced under varied exposure scenarios and at different time points. Such expanded investigations could offer valuable insights into the complex relationship between the environment and macroalgal growth, physiology and development. Several studies have already established optimal growth conditions for *P. palmata* and even reported on some changes in the seaweed's metabolism depending on the exposure scenario (e.g., Corey et al. 2012a, b; Martínez and Rico 2002; Morgan and Simpson 1981). Moreover, the strong influence of seasonality has also been investigated by Vasconcelos et al. (2022), who highlighted variations in the chemical composition of *P. palmata* from Île-de-la-Madeleine (Quebec, Canada) collected over several months. Indeed, dulse had higher protein, mineral, and lipid content during Summer (June and July), whereas carbohydrate content was found to be higher in the Autumn (October). Changes in metabolic pathways may contribute to the tolerance and adaptability of seaweed to different types of stressors (e.g., Park et al. 2023; Wahid and Close 2007). Yet, as far as we know, no studies have focused on exposing *P. palmata* in suboptimal growth conditions (e.g., lower nutrient availability, heat stress), which may boost production of certain compounds within the macroalga as a result. Therefore, the overall aim of the present study was to investigate how changes in several suboptimal cultivation conditions (irradiance, temperature, and nutrient conditions) affect both growth and concentrations of several commonly measured chemical constituents (total fatty acid, lipid, protein, carbohydrate and photosynthetic pigment content) of *P. palmata* in a multivariate experiment. Adaptation of cultivation practices — such as reducing irradiance levels and using lower F/2 nutrient concentrations — can enhance sustainability of the cultivation in tanks. Additionally, elevating temperatures may extend the duration of the growth season at sea, which could further support higher yields. To achieve our aim, we thus conducted a manipulative experiment where the interactive and/or single effects of (1) irradiance and temperature, and (2) irradiance and nutrient levels were tested in indoor cultivation jars with aerated seawater. As such, we aim to provide new insights into optimal growing conditions of *P. palmata* and to elucidate whether these conditions align with achieving an optimal chemical composition. Pinpointing an optimal balance between growth and biochemistry could redefine cultivation strategies for enhanced utility of seaweed aquaculture.

Material & Methods

Palmaria palmata Culture and Origin

Palmaria palmata was reared from tetraspores in the lab from specimens collected in the wild. Mature tetrasporophytes were

collected in April 2022 near Audresselles (50° 50' 0.479" N, 1° 35' 7.914" E, France) and transported back to the laboratory in natural seawater. Tetrasporophytes were cleaned as much as possible from epiphytes by several cycles of rinsing with filtered seawater (5 µm) and wiping with paper towels. After the cleaning, the mature tetrasporophytes were incubated at 12 °C, a short photoperiod (8:16 h light: dark) and cool white light at 20 µmol photons m^{-2} s^{-1} (Osram lumilux, 18 W/840) in a 30 L container filled with 25 L filtered (5 µm) natural seawater enriched with 5 mL of F/2 medium (Guillard and Ryther 1962) for 6 days. The filtered natural seawater, collected at 51.321614 N, 3.143153 E (latitude, longitude, WGS 84) near the high tide line of the coast of Blankenberge (Belgium) was UV treated prior to filtration. After 6 days, released tetraspores were visibly attached to the bottom of the cultivation container and tetrasporophytes were removed. The attached tetraspores were rinsed with clean seawater to remove contaminants as much as possible and kept under the described cultivation conditions. Seawater and medium were renewed every 14 days. Once tetraspores germinated and grew into individuals larger than 0.5 cm, they were detached and transferred into tumbling cultures, incubated under the same conditions. As we did not incubate any males with the released tetraspores, the tetraspores culture resulted in only male macroscopic individuals.

For the experiment, the male gametophytes were then propagated vegetatively (clonally) by separating young thalli from the 'parent' thallus and cultured in F/2 medium with minor adaptations (Supplementary Table 1). This F/2 medium was also refreshed weekly, and was based on filtered natural seawater, filtered over a 0.45 µm filter (Type: polyethersulfon membrane; Acrodisc; Supor 450). The algae were cultivated in flasks (volumes of 2 to 5 L), standing on white cardboard to facilitate light reflection, under an irradiance of 19 µmol m^{-2} s^{-1} (using an overhead TL lamp, Osram lumilux (cool white), 18 W/840, 1300 lm) at a light-dark period of 12:12 h. Cultures were aerated, with aeration being provided through glass Pasteur pipettes, equipped with a bacterial filter, and placed in a culture room at 12 ± 1 °C.

2.2 Setup of the Experiment

Eight *P. palmata* thallus fragments, each about 1–2 cm in length, originating from eight different thalli, were added to 18 Weck jars each. The fragments were distributed so that each Weck jar contained one fragment from each thallus, ensuring intraspecific variability was accounted for. The jars were filled with 1.2 L of F/2 medium and had an average seaweed density ± SD of 1.54 ± 0.26 kg m^{-3}. These macroalgae were exposed to a combination of two irradiance intensities (14 and 19 µmol m^{-2} s^{-1}) and three different temperatures (12, 15 and 17 (± 1) °C) at neutral days (light-dark period of 12:12 h) in temperature-controlled rooms for 12 weeks. Temperatures were chosen based on studies that report the temperature dependence of growth rates of dulse. Cultivation studies of wild-collected material indicate that the optimal growth temperature ranges between 6 and 12 °C, depending on the strain's origin (Morgan and Simpson 1981; Corey et al. 2012a). Reduced productivity has been observed above 14 °C (Corey et al. 2012a), with some studies indicating almost no growth for *P. palmata* at 17 °C (Corey et al. 2012a, 2014). Based on the findings of Schmedes et al. (2020), who investigated impact of irradiance levels between 10 and 280 µmol photons m^{-2} s^{-1} on *P. palmata* growth rates, we opted here for exposure to low irradiance levels (14 and 19 µmol m^{-2} s^{-1}), which are less optimal in terms of growth. A relatively small difference in irradiance was chosen here, as we assumed these levels would already affect photosynthetic pigment production and/or pigment content; and drastic changes in light intensity have already been demonstrated to affect phycobiliprotein production in red algae in literature. Other studies reported exposure of *P. palmata* to significantly higher irradiance levels of 100 to 1,460 µmol photons m^{-2} s^{-1} (Corey et al. 2014), or 125 µmol photons µmol photons m^{-2} s^{-1} (Corey et al. 2012a). Three biological replicates were applied for each treatment (6 treatments, 2 × 3 design). In six additional Weck jars, also containing eight *P. palmata* thalli, only half of the recommended amount of nutrients was administered (termed 0.5 F/2 medium below), and exposed to the treatment at 12 °C (3 replicates *per* light intensity), resulting in the design presented in Fig. 1.

Growth medium was renewed every week. Weck jars were always placed in a random arrangement after refreshing the medium, as depicted in Fig. 1. Jars were aerated in the same way as the stock culture. From week 5–8, Weck jars were filled with 1.5 L of F/2 medium to ensure enough space for continuous growth of the macroalgae. From week 9 onwards, the macroalgae were grown in 2 L aquaria to avoid potential effects of increased density. In a preliminary test, nitrate and phosphate uptake was monitored for both nutrient treatments and across the evaluated temperatures, using two different *P. palmata* densities: the initial tested density (1.54 kg m^{-3}) and the maximum density reached at week 8 (8.5 kg m^{-3}). Less than 40% of the initial nutrient concentrations were consumed in all treatments. Since nutrient depletion did not occur by the end of the week, we assume that depletion was also unlikely during the cultivation experiment at these densities.

At week 12, macroalgae were harvested, stored at −80 °C for 48 h, and subsequently freeze-dried by a FreeZone 4.5 L Benchtop Freeze Dry System (LabConco; −50 °C condenser temperature, vacuum pump 0.200 mbar) for 48 h.

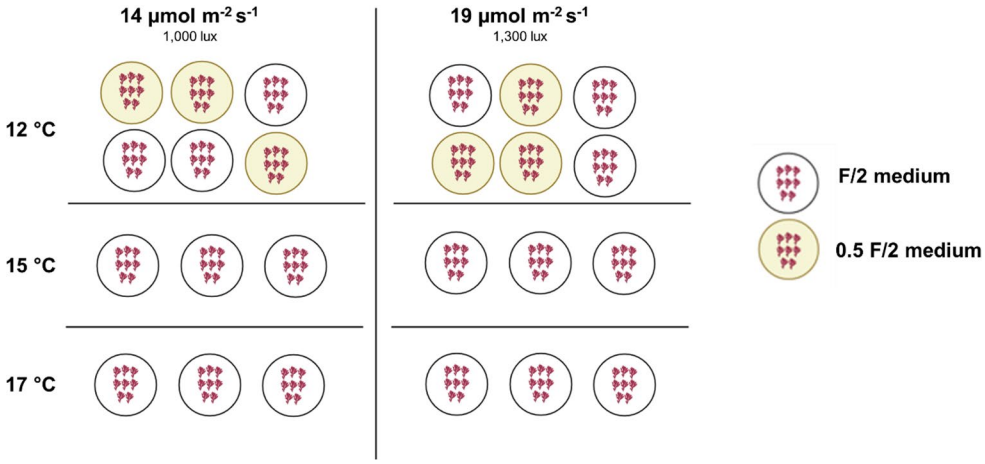

Fig. 1 Figure depicting the experimental setup. 18 Weck jars containing, 8 *Palmaria palmata* specimens each, were placed under three different temperature treatments (12, 15, and 17 (± 1) °C) and two different light intensities (14 and 19 µmol m^{-2} s^{-1}). Additionally, six Weck jars were administered only half of the nutrients as compared to the other jars, as represented by the yellow colour. The position of the Weck jars was randomised and differed every week

After freeze-drying, *P. palmata* was milled into powder using a coffee grinder (Electric Coffee Mill Proficook PC-KSW 1021 N) and stored in 20 mL glass GC vials in desiccators, kept at room temperature.

Biomass Determination

Every two weeks, macroalgal wet weight was determined, which included the first and final week of the experiment. Relative growth rates (RGR, in day^{-1}) were calculated as the change in fresh tissue biomass throughout the experiment:

$$RGR = \frac{\ln W_2 - \ln W_1}{t_2 - t_1} \quad (1)$$

with W_1 and W_2 representing *P. palmata* wet weights (per jar) at times t_1 (start of the experiment) and t_2, respectively.

Fourier-transform Infrared Spectroscopy Analysis

FTIR spectra of freeze-dried and powdered macroalgal material were recorded with a Thermo Scientific Nicolet iN10 MX Infrared Imaging spectrometer (Nicolet Instrument Co., Madison, USA), equipped with a Smart iTR attenuated total reflectance (ATR) sampling accessory. Spectra were scanned between 4,000 and 600 cm^{-1} at room temperature. For each sample, 32 scans were averaged at a resolution of 4 cm^{-1}, prior to processing the obtained IR spectra using the OMNIC software (Nicolet, Madison, USA). Spectra of the samples were normalised for noise: background was scanned 32 times as well. Spectra were visualized using Spectragryph v1.2.16.1. Identification of characteristic peaks was based on literature from Jiao et al. (2012), Nandiyanto et al. (2019), Niemi et al. (2023), Pachetti et al. (2020), and Wu et al. (2015).

Nitrogen and Protein Content

Total nitrogen content was estimated based on the Kjeldahl method (Kjeldahl 1883). Digestion of 50 mg seaweed powder (per sample) was performed with Automat K-438 equipment from Büchi Labortechnik AG (Flawil, Swiss) in the presence of 96% concentrated sulphuric acid (18 mL) and a catalyst. Released ammonia was distilled in a Distillation Unit K-360 (Büchi Labortechnik AG, Flawil, Swiss) and collected in a solution of 4% boric acid, which was subsequently titrated with 0.2 M hydrochloric acid. Two blanks (empty destruction tubes) were added. Subsequently, crude protein content of the samples could be estimated by applying a conversion factor (CF) to the total nitrogen content. Two conversion factors were considered: one for red macroalgae in general (3.99) and one for *P. palmata* specifically (4.10), both obtained from Biancarosa et al. (2017).

Total Lipid Content

Proximate total lipid content in the freeze-dried powder samples was obtained via the Bligh and Dyer (1959) methodology. Briefly, 20 mg of dried seaweed powder was placed into 2 mL microtube in contact with a 1 mL chloroform: methanol (1:1 v/v) mixture. Samples were vortexed (L24 Labinco Power Mixer) and centrifuged (microtube Centrifuge 5424/R) for 10 min at 2,415 rcf and 4 °C. Thereafter, the top fluid phase in the microtube was discarded. From each sample, three times 100 µL from the chloroform layer (containing the lipids,) were taken and put in clean 5 mL glass tubes. The samples were washed with 300 µL of chloroform and vortexed. The chloroform layer was removed by drying the samples in an oven (Thermo Scientific Digital Dry Baths/Block Heaters) at 60 °C for 30 min in a metal rack. Thereafter, 500 µL of sulphuric acid

was added, and the samples were vortexed and dried in an oven (Gelman Instrument Company) at 200 °C for 15 min in a metal rack. After cooling the samples for 15 min, 1.5 mL of deionized water was added, and the samples were stirred (vortexed) again. From each tube, 250 µL was put in a 96 well plate. Optical density was measured at 340 nm using a Thermo Multiskan Ascent microplate reader at an absorbance of 340 nm. Total lipid content was quantified through a calibration curve (R^2 = 0.9952) of a 1:2 serial dilution of a 6.25 mg mL^{-1} tripalmitin standard concentration. Three blanks (chloroform) were run simultaneously with the samples. Total lipid content was expressed by the percentage of dry weight.

Fatty Acid Content

Samples of 100 mg of freeze-dried seaweed powder were analytically weighed and extracted with 5 mL of (2:1) chloroform: methanol (v: v) in a 10 mL glass tube. After vortexing (1 min, L24 Labinco Power Mixer), shaking (15 min, VWR Incubating Mini Shaker), ultrasonicating (5 min, VWR Ultrasonic Cleaning Bath) and centrifuging (5 min, 2,800 rcf, Thermo Scientific Megafuge 40R Refrigerated Centrifuge) the samples, the supernatant was transferred into a 10 mL glass tube. The extraction procedure was repeated two more times with 3 mL (2:1) chloroform: methanol (v: v). The three extracts were collected in the glass tube. Two blanks were included in the extraction. From three samples, a duplicate was taken as technical replicate. The samples were then dried with a Techne DB200/3 Digital Dri-Block heater at 50 °C and under nitrogen gas in the fume hood.

To each of the evaporated extracts in the glass tubes, 25 µL of internal standard (palmitic acid D31–2,000 ppm, CAS 39756-30-4) was added. After vortexing (1 min, L24 Labinco Power Mixer), 3 mL of 3 M HCl in MeOH (Sigma-Aldrich) was added. The samples were vortexed again and placed in a Techne DB200/3 Digital Dri-Block heater (60 °C) for 15 min in the fume hood. After cooling the samples, three extractions were performed with 100% hexane (3 mL). After each extraction, the supernatant was transferred into a second glass tube. A centrifugation step (5 min, 1,000 rcf) was applied when the phases were not properly separated. The volume of the three fractions combined in the second glass tube was brought to 10 mL with hexane. In addition, two blanks were incorporated into the derivatisation step. Eventually, the tubes were stored at 4 °C until analysis.

P. palmata extracts were subjected to Gas chromatography–mass spectrometry (GC–MS) analysis to analyse fatty acid content and composition. Analyses were performed on a Thermoquest Trace-GC connected to a Thermoquest DSQ-MS and equipped with a DB-225 ([50%-cyanopropylphenyl]-dimethylpolysiloxane, 30 m × 0.25 mm, 0.250 µm film thickness) column. The active components were separated using helium as carrier gas at a flow rate of 1 mL min^{-1}. Samples (1 µL) were injected in splitless mode at 200 °C. The following oven settings were applied: initial temperature of 50 °C held for 1 min; followed by increasing to 185 °C at a rate of 7 °C per minute, held for 10 min; then increased to 230 °C at a rate of 15 °C per min, which was held at 230 °C for 14 min. The transfer line to the mass detector was set at 240 °C, the ion source at 250 °C. Detection was performed in full scan mode (50–440 m/z) in electron impact mode (70 eV). X-Calibur was used for chromatogram acquisition and processing. Identification of the fatty acids was based on certified reference materials, and the yield of the internal standard was 78%. The limit of detection (LOD) was calculated from the limit of quantification (LOQ). Concentrations smaller than the LOD were omitted from further analysis. Concentrations between LOD and LOQ were set as the average of LOD and LOQ in data treatment.

Carbohydrate Content

Neutral monosaccharides were determined as alditol acetates via gas chromatography with a flame ionization detector (GC-FID), using 2-deoxyglucose (1.0 mg mL^{-1}) as internal standard, following Nunes et al. (2012), Oliveira et al. (2017), and Pandeirada et al. (2019) protocols. Samples were subjected to a pre-hydrolysis with 0.2 mL of 72% of H_2SO_4 (w/w) for 3 h at room temperature followed by 2.5 h hydrolysis with 1 M of H_2SO_4 at 100 °C. Three glass spheric beads (0.5 cm in diameter) were added to each tube to promote further disruption of the samples. Neutral sugars were converted into their alditol acetates after reduction with $NaBH_4$ (15% (w/v) in 3 M of NH_3) at 30 °C for 1 h and acetylation with acetic anhydride in the presence of 1-methylimidazol, as a catalyst, at 30 °C for 30 min. A gas chromatograph with flame ionization detector (FID; Perkin-Elmer Clarus 400, Massachusetts, USA) was used to separate, identify, and quantify the formed alditol acetates. The instrument was equipped with a 30 m x 0.25 mm DB-225 capillary column (Agilent J&W Scientific, Folsom, CA, USA) with a film thickness of 0.15 µm. The operating temperatures of the injector and the detector were 220 °C and 230 °C, respectively. The GC oven temperature program was set for an initial temperature of 200 °C for 1 min, raised to 220 °C at 40 °C min^{-1}, holding for 7 min at this temperature, then raised to 230 °C at 20 °C min^{-1}, and maintained for 1 min. The flow rate of the carrier gas

(H_2) was set at 1.7 mL min^{-1}. The analysis of all samples was performed in triplicate. A fourth analysis was done for the few samples with higher variability. The total content of carbohydrates was determined through the sum of the monosaccharide contents.

The presence of uronic acids (UAs) within the seaweed polysaccharides was evaluated as well. UAs were quantified by a modification (as described in Ferreira et al. 2020; Nunes et al. 2008) of the 3-phenylphenol colorimetric method (developed by Blumenkrantz and Asboe-Hansen 1973). Samples were prepared by pre-hydrolysis in 200 µL of 72% of H_2SO_4 for 3 h at room temperature followed by hydrolysis with 1 M of H_2SO_4 at 100 °C for 1 h. Three glass spheric beads (0.5 cm in diameter) were added to each tube to promote further disruption of the samples. A calibration curve (R^2 of 0.98) was made with D-galacturonic acid in a concentration series ranging between 0 and 80 µg mL^{-1}. The absorbance of both the standards and samples was read at 520 nm using an absorbance microplate reader (EON, BioTek, USA).

R-Phycoerythrin Content

Briefly, R-phycoerythrin was extracted from the freeze-dried biomass using a solid-liquid ratio of 0.07 g dry seaweed powder *per* mL solvent (1 M of phosphate buffered saline [PBS] solution, pH 7.4) in duplicate *per* sample. The extraction was performed for 30 min at room temperature in a vertical trayster (IKA KS 4000 IC control) at 80 rpm and protected from light with aluminium foil. Afterwards, the extracts were centrifuged at 12,000 rcf, for 5 min and at room temperature in a VWR Microstar 17 centrifuge. After filtration (0.45 µM, Nylon filter), the supernatant was used for phycobiliprotein quantification. Extraction of the residue was repeated to confirm the exhaustion of pigments. The absorbance spectra of 200 µL of the aqueous phases were measured between 300 and 700 nm using a UV−Vis microplate reader (Synergy HT microplatereader−BioTek) and absorbance was quantified at 564 nm for R-phycoerythrin. R-phycoerythrin content was evaluated according to a calibration curve determined for the project (R^2 of 0.9996).

Total Antioxidant Activity

Total antioxidant activity of macroalgae extracts was quantified as described in Prieto et al. (1999) and Meenakshi et al. (2011). The method is based on the reduction of Mo(VI) to Mo(V) of the sample and the subsequent formation of a green phosphate/Mo(V) complex at acidic pH. Briefly, 20 mg of freeze-dried seaweed powder of each sample was extracted in 1 mL of aqueous methanol and placed in a VWR Incubating Mini Shaker for 1 h at room temperature in the dark. After centrifugation at 10,000 rcf for 15 min, an aliquot of 0.1 mL of the supernatant was combined in a microtube tube with 1 mL of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The microtubes were incubated in a thermal block (Thermo Scientific Digital Dry Baths/Block Heaters) at 95 °C for 90 min, and subsequently cooled to room temperature. Finally, 250 µL of each sample was then pipetted into a multi-well plate to be measured at an absorbance of 695 nm with a Thermo Multiskan Ascent microplate reader. In addition to the inclusion of a pure methanol blank, a blank solution containing 1 mL of reagent solution and 0.1 mL methanol was also included. A calibration curve (ascorbic acid in methanol in the following concentrations 1.0, 0.5, 0.25, 0.125, 0.0625, 0.03125 and 0.015625 mg mL^{-1}; R^2 = 0.9971) was constructed to calculate the concentration of antioxidants in the samples. The results were expressed as mg of ascorbic acid equivalent per g DW.

Data Treatment and Statistical Analysis

Measurements of each parameter are expressed as the mean ± standard deviation. Statistical tests were executed in R v4.2.2, implemented in RStudio v.2023.03.0+386. Prior to data analysis, the data was checked for the presence of outliers. The Shapiro-Wilk test of normality (stats package v.4.2.2) and Levene's test of homogeneity of variances (car package v.3.1-2.1) were applied to verify analysis of variance (ANOVA) assumptions. Differences in chemical composition of *P. palmata* were statistically analysed using ANOVA (Two-way, stats package) or non-parametric alternatives Kruskal-Wallis and Wilcoxon tests (stats package). These analyses were followed by appropriate post-hoc analysis for pairwise comparisons (Tukey HSD test for parametric data and Dunn's test for nonparametric data). Adjustment for multiple testing was performed *via* the Bonferroni correction. Results were considered statistically significant if the obtained p-value was less than 0.05. Graphs were constructed using the ggplot2 package v.3.4.0. Finally, a principal component analysis (PCA) analysis was performed using the factoextra package v.1.0.7.

Human Health Potential of P. palmata Consumption

Several nutritional indices based on fatty acid composition were determined as they are commonly used to assess the nutritional quality of marine food products (Chen and Liu 2020). The atherogenic index (AI) and thrombogenic index

(TI) were considered to assess lipid composition in relation to coronary heart disease risk factors. AI and TI were calculated according to Ulbricht and Southgate (1991):

$$AI = \frac{[C12:0+(4\times\ C14:0)+C16:0]}{\sum UFA} = \frac{[C12:0+(4\times\ C14:0)+C16:0]}{\sum MUFA+\sum PUFA(n-6)+\sum PUFA(n-3)} \quad (2)$$

$$TI = \frac{[C14:0+C16:0+C18:0]}{0.5\sum MUFA+0.5\sum PUFA(n-6)+3\sum PUFA(n-3)+\frac{\sum PUFA(n-3)}{\sum PUFA(n-6)}} \quad (3)$$

With UFA referring to Unsaturated Fatty Acids, MUFA representing Monounsaturated Fatty Acid (a type of UFA with one double bond), PUFA denoting Polyunsaturated Fatty Acid (a type of UFA with two or more double bonds), ∑PUFA(n-6) being total omega-6 content and ∑PUFA(n-3) representing total omega-3 content.

The AI indicates the relationship between the sum of saturated fatty acids (SFAs), which include C12:0, C14:0, and C16:0, and the sum of main classes of unsaturated fatty acids (UFAs). The former are considered pro-atherogenic (i.e., favouring the adhesion of lipids to cells of the immunological and circulatory system), while the latter are considered anti-atherogenic (i.e., inhibiting the aggregation of plaque and diminishing the levels of esterified fatty acids, cholesterol, and phospholipids, hence, preventing the appearance of coronary diseases) (Chen and Liu 2020; Ulbricht and Southgate 1991). The TI indicates the tendency to form clots in blood vessels and is defined as the relationship between the pro-thrombogenic (saturated) and the anti-thrombogenic FAs (MUFAs and the n-3 and n-6 families) (Chen and Liu 2020; Ulbricht and Southgate 1991).

Results

3.1. Impact of temperature, irradiance, and Nutrient Load on P. palmata Growth Rates

The effect of temperature and irradiance on the RGR of *P. palmata* specimens (grown in F/2 medium) was analysed every two weeks (Suppl. Figure S1; Suppl. Table S2), with only temperature having a significant impact at weeks 2, 4, 6, 8, 10 and 12 (ANOVA, $p<0.001$), as visualised in Fig. 2 for week 12 (A). Post hoc analysis indicated significant differences in RGR between all temperatures from week 8 onwards, regardless from irradiance (Tukey HSD test; $p<0.05$). At weeks 4 and 6 there was no significant difference in RGR between the 15 and 17 (±1) °C treatments (Tukey HSD test; $p>0.05$). Similarly, the RGR, averaged over the entire run of the experiment, was significantly higher at 12 °C than in the treatment groups exposed to 15 and 17 (±1) °C (Tukey HSD test, Suppl. Figure S1). However, we observed a steady decrease of growth rates over the weeks in all treatments from week 4 onwards (Table S2, Suppl. Fig. S1). Highest average relative growth rate was observed at 12 °C, 14 µmol m^{-2} s^{-1} in F/2 medium, while the lowest average relative growth rate was observed at 17 °C, 14 µmol m^{-2} s^{-1} in F/2 medium. ANOVA analysis revealed a significant effect ($p<0.01$) of temperature on harvested biomass (wet weight), grown in F/2 medium, as well as an interaction effect of temperature and irradiance (Fig. S2 in Supplementary Information). Harvested biomass was significantly lower with increased temperatures 15 and 17 °C, compared to the 12 °C treatment, grown in full F/2 medium (Tukey HSD test, $p<0.05$). As a result, at week 12, the average seaweed density in the 2 L aquaria kept at 12 °C

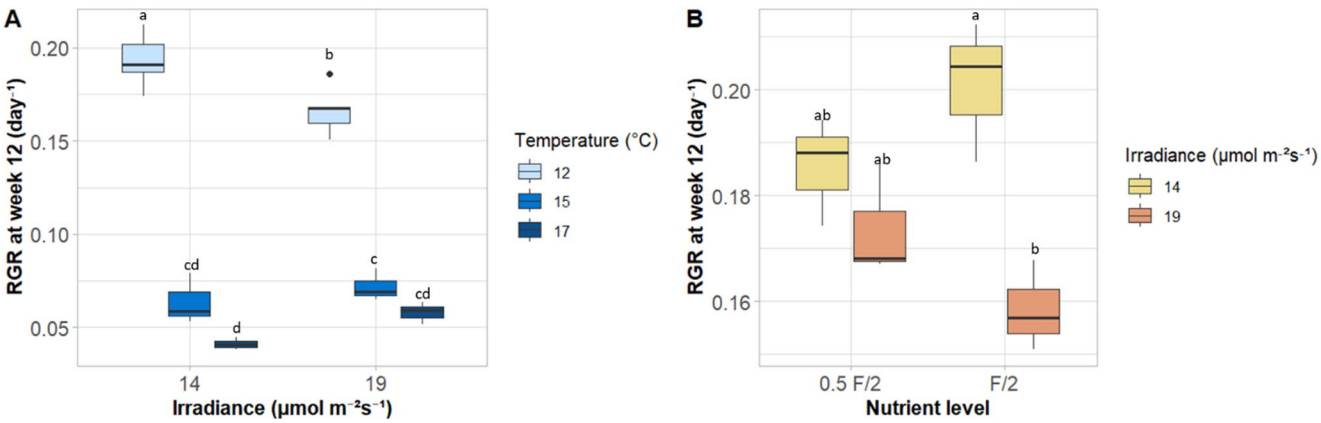

Fig. 2 (**A**) Effect of temperature and irradiance on relative growth rate (RGR) at week 12, cultivated in regular F/2 medium. (**B**) Effect of irradiance and nutrient supply on *Palmaria palmata* RGR at week 12, at a water temperature of 12 (±1) °C. Abbreviations used: F/2 = normal nutrient supply, i.e., in regular F/2 medium; 0.5 F/2 = lower nutrient supply, only half of nutrients were administered compared to the standard levels. The horizontal bold line in each box represents the median, the box itself represents upper and lower (25% and 75%) quartiles, the bars encompass the data range, while dots placed past the bar edges indicate outliers. Different letters indicate statistically significant differences (adjusted $p<0.05$)

(6.62 ± 1.48 kg m^{-3}) was significantly ($p < 0.05$) higher compared to those kept at 15 and 17 (±1) °C (1.80 ± 0.42 kg m^{-3} and 1.40 ± 0.22 kg m^{-3}, respectively). Some of the tips of the *P. palmata* fronds exposed to higher temperatures (i.e., 15 °C, 17 °C) displayed green discoloration at the time of harvest, instead of their distinct red hue (Fig. S3 in Supplementary Information).

The effect of irradiance and nutrient supply on RGR (cultivated at a temperature of 12 (±1) °C) from week 4 onwards yielded a significant difference between the irradiance groups (ANOVA, $p < 0.01$), with a lower RGR in the higher irradiance group (Tukey HSD, p adj. <0.05), regardless of nutrient treatment. Moreover, post hoc analysis revealed a significant higher RGR in *P. palmata* grown in F/2 medium under an irradiance of 14 µmol m^{-2} s^{-1} compared to the group exposed to 19 µmol m^{-2} s^{-1} in F/2 medium (Tukey HSD, p adj. <0.05), but this pattern was not repeated in the 0.5 F/2 treatment, as shown in Fig. 2B for week 12. In contrast to the following weeks, at week 2, a significant lower RGR was observed for *P. palmata* grown under lower nutrient conditions compared to the F/2 treatment, regardless of irradiance (Tukey HSD, p adj. <0.05). While an insignificant impact of nutrient supply was found in any other week, the interaction between irradiance and nutrient supply yielded a significant effect at weeks 10 and 12 (ANOVA, $p < 0.05$). ANOVA analysis revealed a significant effect ($p < 0.05$) of irradiance and nutrient supply on harvested biomass (fresh weight), but no interaction effect (Fig. S2 in Supplementary Information). Harvested biomass was significantly lower under higher irradiance, as well as under lower nutrient supply at 12 °C (Tukey HSD test, $p < 0.05$).

Qualitative Screening of Differences in Chemical Composition of P. palmata Via ATR-FTIR Analysis

Figure 3 illustrates the FTIR spectra of freeze-dried and powdered *P. palmata* for all treatments and replicates. All spectra displayed a similar absorbance pattern, and no additional peaks or losses were observed across the treatments, suggesting limited number of gains or losses in compound diversity. The main peaks with their corresponding functional groups were determined. The broad peaks centred at 3550–3200 cm^{-1} (peak 1 on Fig. 3) can be assigned to hydrogen-bonded O-H and N-H stretching vibrations, corresponding to the presence of polysaccharides and amino acids. The peak around 2930 cm^{-1} (2) was caused by the C-H asymmetric stretching on saturated carbon atoms, suggesting the existence of aliphatic compounds. The peaks around 2350 cm^{-1} (3&4) are caused by the presence of O=C=O stretching of carbon dioxide. The strong C=O stretching around 1630 cm^{-1} (5) suggests the existence of COOH groups. The N-H stretching around 1547 cm^{-1} (6) indicates the presence of amino acids. The absorbance around 1400 cm^{-1} (7) indicates the presence of carboxylic acid (C=O). The presence of acetate ($C_2H_3O^-_2$) groups could cause the absorbance peak at 1348–1349 cm^{-1} (8). Peaks in the spectra around 1250 cm^{-1} (9) can be assigned to sulphate groups (S=O) and peaks around 1030 cm^{-1} (10) are probably due to the C-O binding stretch of the polysaccharides. Absorbances at 1070 cm^{-1} indicate vibrations of C-OH bonds of galactose. The small peak around 900 cm^{-1} is likely caused by β-glycoside bonds (C–O–C), which confirm that sugar units linked together in the polysaccharide structures are present in the biomass (11).

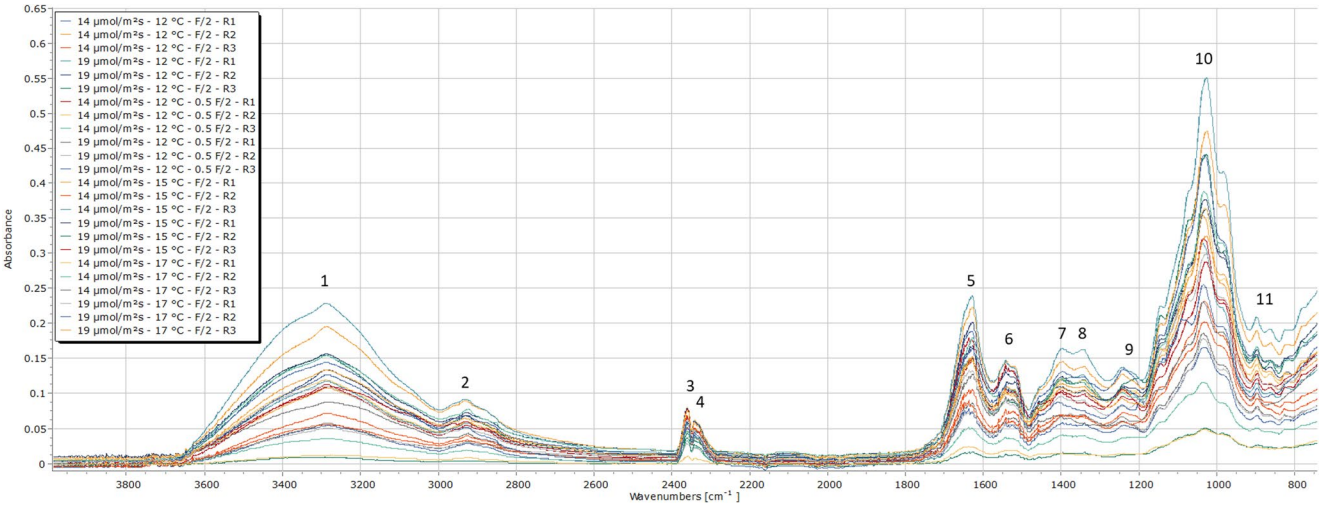

Fig. 3 FTIR spectra of all freeze-dried and powdered *Palmaria palmata* samples

Impact of Temperature and Irradiance on P. palmata Chemical Composition

Regardless of the treatment, *P. palmata* had a mean protein-nitrogen content of 12±1% DW with a CF of 4.10. A pairwise comparison using the Tukey HSD test revealed significant differences in nitrogen content, and consequently in protein-nitrogen content, with lower nitrogen content in the 12±1 °C treatment compared to the 15±1 °C or 17±1 °C groups (Tukey HSD test, adjusted p-value<0.01). These differences are illustrated in Fig. 4A. Moreover, nitrogen content was significantly lower in the 19 µmol m^{-2} s^{-1} treatment, compared to nitrogen content in *P. palmata* grown at a lower irradiance, regardless of temperature exposure (Tukey HSD test, adjusted p-value<0.01).

No significant effects of temperature and irradiance were observed for total lipid content (determined by the colorimetric Bligh & Dyer method, Fig. 4D) in *P. palmata*. Eicosapentaenoic acid, EPA, was the most abundant fatty acid in all treatments, averaging 0.8±0.7 mg g^{-1} DW and representing 38.3% of total fatty acid content (Suppl. Figure S4). The second most abundant FA was palmitic acid, with an average of 0.6±0.3 mg g^{-1} DW, representing 34.1% of the total fatty acid content. In contrast to total lipid content, temperature exhibited a significant effect on the FA content (summed) considering the total FA composition (Kruskal-Wallis test, $p<0.01$), as well as myristic acid, linoleic acid (Kruskal-Wallis, $p<0.01$; Fig. S4), EPA and palmitic acid content (ANOVA, p-value<0.01; Fig. S4). Regardless of irradiance, post-hoc analyses demonstrated a significant higher total of FA, myristic acid, stearic acid, palmitic acid and EPA contents at 12 °C compared to the 15 °C and 17 °C treatments (Tukey HSD or Dunn's test, adj. p-value<0.05), while linoleic acid amount was only significantly different between 12 and 17 °C (Dunn's test, adj. p-value<0.05). The essential omega-3 fatty acid docosahexaenoic acid (DHA) was not recovered in any of the samples. Table S3 from Supplementary information provides an overview of the fatty acid composition of *P. palmata* grown in these different conditions.

Table 1 shows total sugar content (mg g^{-1} DW) and molar ratios of the monosaccharide residues in the total carbohydrate content for the different treatments. The carbohydrate content determined in this study averaged 467±52 mg g^{-1} DW. We found significantly higher galactose levels in *P. palmata* grown at 12±1 °C versus *P. palmata* grown at 15±1 or 17±1 °C (Tukey HSD, adjusted p-value<0.05), while for xylose content exactly the opposite trend was observed, regardless of irradiance (Fig. 5). Both glucose and total sugar content were only significantly higher in *P. palmata* exposed to 17±1 °C versus 15±1 °C, regardless of irradiance (Tukey HSD, adjusted p-value<0.05). In contrast, irradiance was only found to significantly affect galactose content in *P. palmata*, with higher galactose levels found in the 19 µmol m^{-2} s^{-1} group, regardless of temperature (Tukey HSD test, adj. $p<0.05$). Comparing all different treatments however, no significant differences in total sugar content were found (Tukey HSD, adjusted p-value>0.05). UAs were not present or were below the detection limit. We found an average concentration of R-phycoerythrin of 657±201 µg g^{-1} DW (all treatments considered). Temperature exhibited a significant effect on R-phycoerythrin content (ANOVA, p-value<0.01) in *P. palmata* cultured at 12±1 °C compared to higher temperatures at a lower irradiance (Tukey HSD test, adj. $p<0.05$; Fig. 4), but different irradiance levels did not. Similarly, a significant higher antioxidant content was observed in *P. palmata* grown in 12±1 °C versus seaweed grown at higher temperatures at a higher irradiance (Tukey HSD test, adjusted p-value<0.01).

Impact of Nutrient Load on P. palmata Chemical Composition

Table S4 from Supplementary Information presents a summary of the fatty acid composition of *P. palmata* cultivated under the different nutrient treatments at 12±1 °C, while monosaccharide composition is presented in Table 1. No significant effects of nutrient supply and irradiance were observed for total carbohydrate, galactose, xylose, glucose, and fatty acid content, as well as antioxidant activity in *P. palmata* grown at 12±1 °C (Fig. 6 and Figs. S5 and S6 from Supplementary Information). In contrast, nutrient supply exhibited a significant effect on nitrogen and total lipid content, but irradiance not (ANOVA, p-value<0.05 and p-value<0.01, respectively; Fig. 6). Furthermore, the interaction between nutrient supply and irradiance showed a significant effect on nitrogen content in the seaweed biomass (Fig. 6). Pairwise comparison (Tukey HSD test) revealed significant differences between 0.5 F/2–14 µmol m^{-2} s^{-1} and F/2–19 µmol m^{-2} s^{-1}, 0.5 F/2–19 µmol m^{-2} s^{-1} and F/2–14 µmol m^{-2} s^{-1} and 0.5 F/2–19 µmol m^{-2} s^{-1} and F/2–19 µmol m^{-2} s^{-1} treatments (adjusted p-value<0.05). We did not find any significant difference in R-phycoerythrin content in dulse exposed to different nutrient or irradiance levels, when grown at 12±1 °C as it can be seen in Fig. 6.

Figure 7. Principal component analyses (PCA) and corresponding correlation circle plot based on the first two principal components (PC1 and PC2) generated from the analysed compositional parameters (after log transformation) of the (bioactive) components in *Palmaria palmata* grown at different temperatures (A). Single points refer to an individual seaweed sample grown at a specific temperature, as indicated in the legend. (B) correlation circle plot. Vectors are the loadings on PC1 (x-axis) and PC2 (y-axis). The quality of representation of the variables

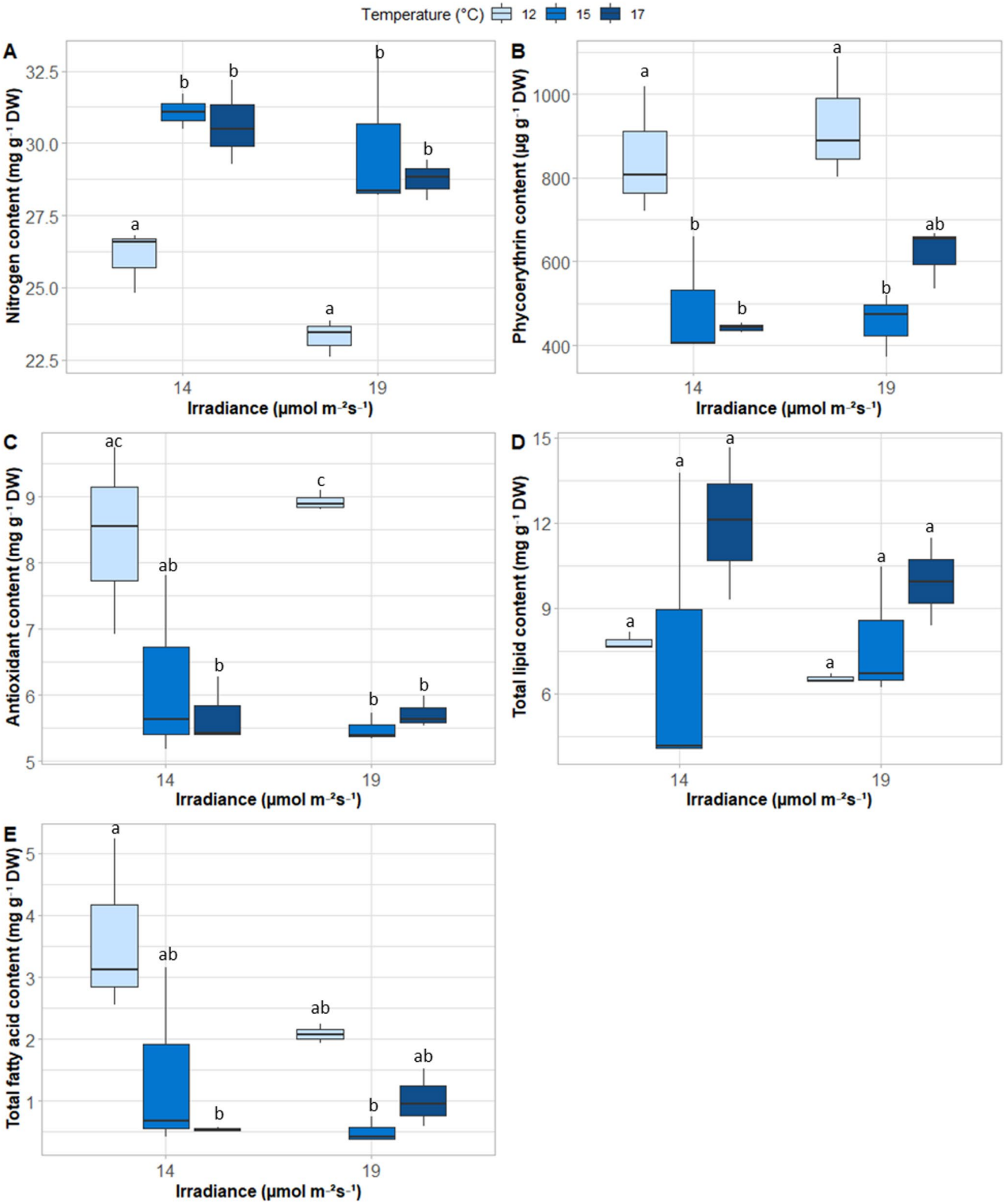

Fig. 4 Box plots illustrating (**A**) nitrogen content, (**B**) R-phycoerythrin content, (**C**) antioxidant content, (**D**) total lipid content, and (**E**) total fatty acid content of *Palmaria palmata* grown under different temperature and irradiance conditions (mg g^{-1} DW). The horizontal bold line in each box represents the median, the box itself represents upper and lower (25% and 75%) quartiles, bars encompass the data range, while dots placed past the bar edges indicate outliers. Different letters indicate statistically significant differences (adjusted $p<0.05$)

Table 1 Average (±SD) monosaccharide residues composition (%mol), and total sugar content (mg g^{-1} DW) of *Palmaria Palmata* biomass grown at different conditions. Abbreviations used: Xyl = xylose, Gal = galactose, Glc = glucose. F/2 and 0.5 F/2 refer to treatments where the full and half of the recommended nutrient concentrations were applied, respectively

Treatment			Yield	Molar ratio (%)		
Temperature (±1 °C)	**Irradiance (µmol $m^{-2}s^{-1}$)**	**Nutrient level**	**Total Sugar content (mg g^{-1}DW)**	**Xyl**	**Gal**	**Glc**
12	14	F/2	437±96	61±8	26±4	13±5
12	14	0.5 F/2	435±27	61±3	28±2	18±2
12	19	F/2	574±110	53±6	32±5	16±2
12	19	0.5 F/2	267±48	51±3	33±2	16±3
15	14	F/2	385±86	71±3	17±2	12±2
15	19	F/2	448±31	72±1	18±1	10±2
17	14	F/2	487±52	69±3	15±2	16±2
17	19	F/2	518±124	61±3	20±1	19±2

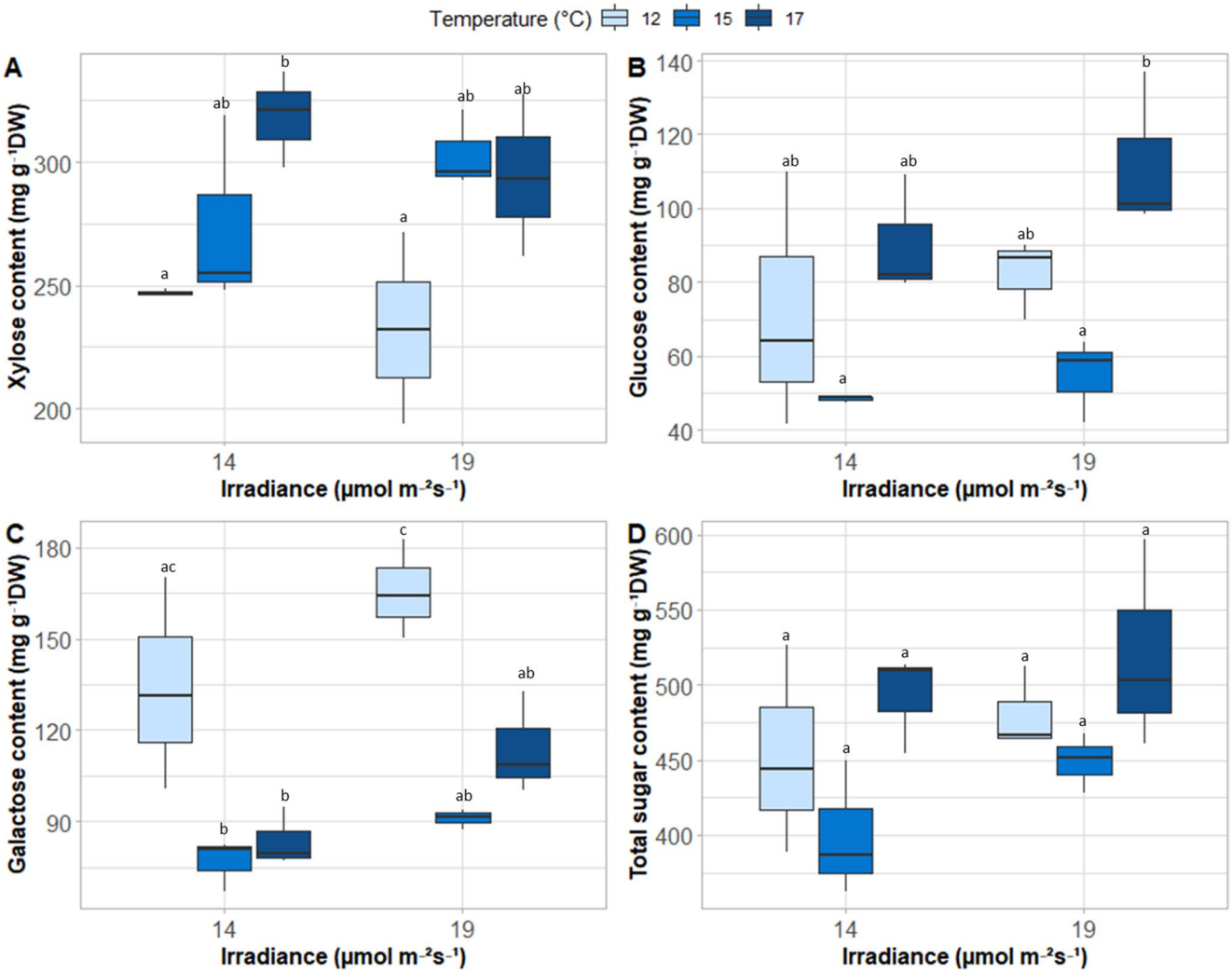

Fig. 5 Box plots illustrating monosaccharide and carbohydrate content of *Palmaria palmata* grown under different nutrient and irradiance conditions (mg g^{-1} DW). (**A**) Xylose content. (**B**) Glucose content. (**C**) Galactose content. (**D**) Total carbohydrate (sum of monosaccharides) content. The horizontal line represents the median, the box itself represents upper and lower (25% and 75%) quartiles, bars encompass the data range, while dots placed past the bar edges indicate outliers. Different letters indicate statistically significant differences (adjusted $p < 0.05$)

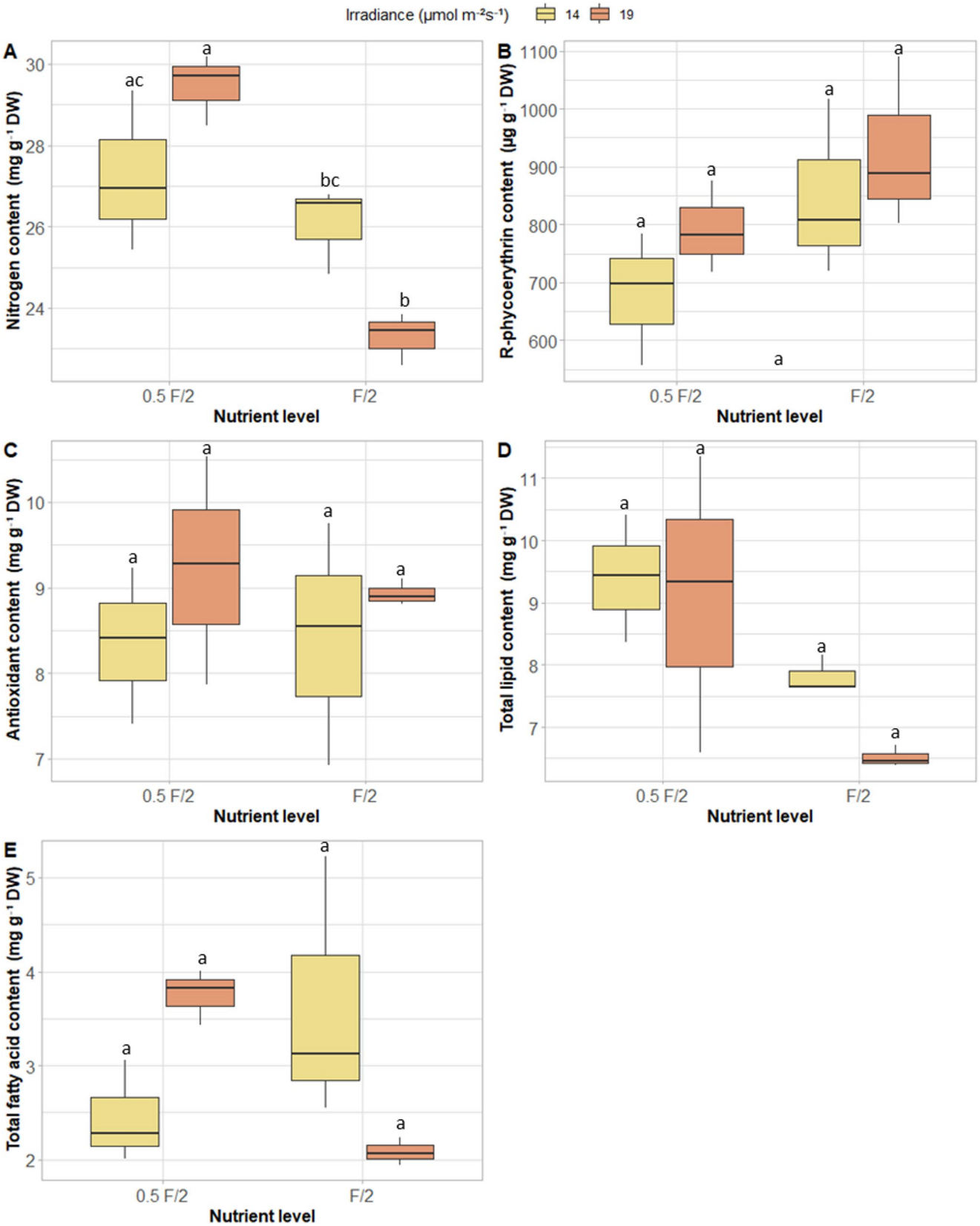
Irradiance (µmol m⁻²s⁻¹) 14 19
A
Nitrogen content (mg g⁻¹ DW)
ac
a
bc
b
0.5 F/2
F/2
Nutrient level
B
R-phycoerythrin content (µg g⁻¹ DW)
a
a
a
a
0.5 F/2
F/2
Nutrient level
C
Antioxidant content (mg g⁻¹ DW)
a
a
a
0.5 F/2
F/2
Nutrient level
D
Total lipid content (mg g⁻¹ DW)
a
a
a
a
0.5 F/2
F/2
Nutrient level
E
Total fatty acid content (mg g⁻¹ DW)
a
a
a
0.5 F/2
F/2
Nutrient level

Fig. 6 Box plots illustrating variation in (**A**) nitrogen content, (**B**) R-phycoerythrin content, (**C**) antioxidant activity, (**D**) total lipid content, and (**E**) total fatty acid content of *Palmaria palmata* grown under different nutrient and irradiance conditions (µg or mg g^{-1} DW). The bold horizontal line in each box represents the median, the box represents upper and lower (25% and 75%) quartiles, bars encompass the data range, while dots placed past the bar edges indicate outliers. Different letters indicate statistically significant differences (adjusted $p<0.05$)

(seaweed components) is indicated by the squared cosine (cos2). A high cos2 value indicates a good representation of the component on the principal component. Vector length indicates the strength of the relationship and the angle between two vectors gives the degree of correlation (adjacent = highly correlated components, orthogonal (90°) = uncorrelated components, and opposite (180°) = negatively correlated components).

Human Health Potential of P. palmata Metabolites

Figure 8 gives an overview of atherogenic and thrombogenic indices (AI and TI, respectively), both calculated for each treatment. Log transformed AI an TI values were significantly lower for *P. palmata* grown in 12 ± 1 °C versus the highest temperatures analysed in this work, regardless of irradiance (Tukey HSD, adjusted p-value > 0.05). We did not observe a significant difference based on differences in nutrient conditions, as well as light irradiance (regardless of other parameters).

Discussion

P. palmata Biomass Yields and Growth Rates

We observed that *P. palmata* displayed the highest growth rate at the temperature of 12 ± 1 °C and lower growth rates were observed when specimens were grown at 15 ± 1 °C and 17 ± 1 °C. Based on cultivation studies of wild-collected material, the optimal growth temperature ranges between 6 and 12 °C (Morgan and Simpson 1981; Corey et al. 2012a) depending on the strain origin. The in situ temperature growth range of *P. palmata* is 6 °C to 17 °C and the growth rate of this seaweed was found to be reduced at temperatures above 15 °C, which corroborates our findings (Grote 2017). Similarly, a lower productivity is reported above 14 °C by Corey et al. (2012a) and increased mortality of the biomass at 21 °C (Matos et al. 2006).

Werner and Dring (2011) proposed an irradiance of 200–400 µmol m^{-2} s^{-1} at a light-dark period of 16:8 h to achieve optimal growth rates for *P. palmata*, however, these were not quantified. These irradiance levels are considerably higher than those in the experimental setup (14 and 19 µmol m^{-2} s^{-1}). In this study, higher growth rates at the higher irradiance treatment (19 µmol m^{-2} s^{-1}) were observed at the more extreme temperatures (i.e., 15 ± 1 °C and 17 ± 1 °C), but these were not significantly different from those at 14 µmol m^{-2} s^{-1}. In contrast, we found a significant higher RGR of *P. palmata* reared at 14 µmol m^{-2} s^{-1} versus 19 µmol m^{-2} s^{-1} for specimens reared at 12 ± 1 °C at week 12, regardless of cultivation medium. Incorporating a higher or optimally adjusted irradiance in our design might have offered further insights into how light availability influences both the yield and chemical composition of *P. palmata*. Pang and Lüning (2004) reported increasing growth rates and yields of *P. palmata* over a wide range of irradiances, with no signs of chronic photoinhibition, up to a growth-saturating irradiance of approximately 1600 µmol m^{-2} s^{-1} in yellowish light.

We observed a decrease of growth rates over the weeks in all treatments when compared to the start of the experiment (Table S2, Fig. S1). Reduced growth rates may also be the result of increased costs associated with maintaining homeostasis and supporting higher body masses. Nevertheless, nutrient supply had a significant effect on the biomass amount (fresh weight), as yields were significantly higher in the F/2 treatment compared to the 0.5 F/2 treatment, regardless of irradiance, although this difference was not reflected in the corresponding RGRs. Nutrients are crucial for the growth and development of seaweed (Gao et al. 2018). In this study, we did not actively monitor the nutrient uptake in the macroalga during the course of the experiment, nor did we quantify the nutrient availability before and after each medium renewal. Consequently, we cannot definitively ascertain whether the macroalga experienced any nutrient limitation during our experiments. We assume nutrient limitation would not pose a problem during the first 8 weeks of the experiment, based on the results from the preliminary test. Nutrient limitation might have occurred at the end of the last weeks due to increased seaweed densities, even though the seaweed was cultivated in bigger aquaria. Regardless, nutrient concentrations fluctuated throughout the experiment because of uptake by the seaweed and weekly renewals. This could have an impact on nutrient uptake kinetics and by consequence on seaweed metabolism. Possible approaches to address changes in nutrient availability include pruning or cutting the seaweed to maintain stable densities in each treatment, or adding extra nutrients to the medium based on achieved seaweed density on frequent occasions (e.g., daily renewal or via an adjusted flow through set up). However, both methods are challenging to implement and may introduce further alterations in seaweed chemistry. For instance, pruning or cutting could trigger anti-predator chemical defenses in the seaweed (e.g., Hay et al. 1987; Toth and Pavia 2007) due

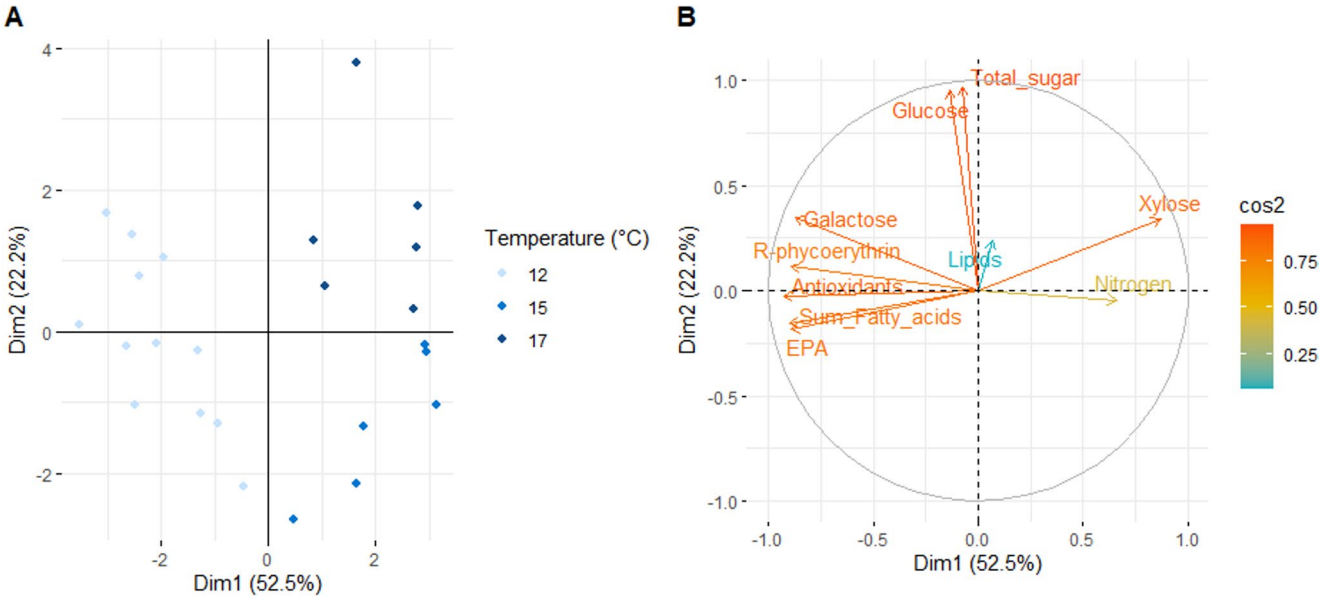

Fig. 7 shows a PCA of variables for specimens cultivated at different temperatures, light intensities and nutrient levels, based on the respective concentrations (mg g^{-1} DW) of each compound. Components one and two explain 74.7% of the total variability. The PCA of variables indicated a negative correlation between nitrogen and R-phycoerythrin content (R of −0.72, Pearson, p-value < 0.01). Similarly, glucose and total carbohydrate content clustered together and formed a 90° angle with xylose, meaning xylose content is unrelated to glucose content. R-phycoerythrin, sum of fatty acid and galactose content clustered together, demonstrating a high degree of correlation

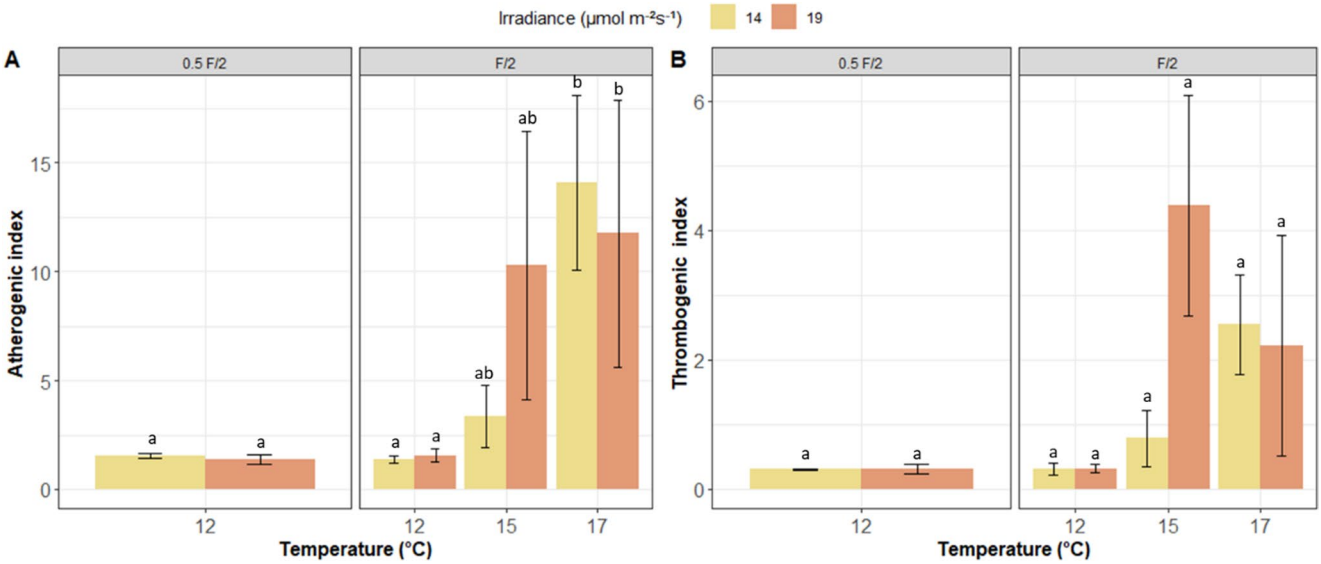

Fig. 8 Bar plots illustrating average (±SD) values of (**A**) Atherogenic index (AI), and (**B**) Thrombogenic index (TI) calculated based on fatty acid profiles of *Palmaria palmata* grown under different temperature, nutrient and irradiance conditions. Different letters indicate statistically significant differences per condition (Tukey HSD test on log transformed data, adjusted $p < 0.05$)

to tissue damage, while adjusting nutrient levels by density would require frequent seaweed handling to maintain nutrient concentrations in the medium. Nutrients were found to be rapidly absorbed when seaweed is nitrogen-starved for storage in its tissue, leading to the quick depletion of nutrients in the medium, as seen with *Gracilaria tikvahiae* (Ryther et al. 1981). Similar nutrient uptake dynamics may take place in cultivated seaweed that is "pulse-fed" on a regular basis. Future research should thus delve further into nutrient uptake dynamics to better understand how the biochemical composition might be influenced under varied nutrient availabilities and at different time points. Moreover, we want to highlight that nutrient uptake by red seaweed is not only a function of external nitrogen concentration, but also of the internal concentration in its tissues (e.g., through storage in phycobiliproteins for example). Finally, adjusting

nutrient supplementation does not address potential shading effects arising from increased densities, which may also influence seaweed physiology. We therefore want to emphasize the importance of considering these factors when cultivating seaweed or reporting its chemical composition.

Nitrogen and Protein Content of P. palmata

Regardless of all treatments, the nitrogen content was found to be 2.8 ± 0.3% DW, comparable to the findings of Chaumont (1978) and Martínez and Rico (2008), where maximum nitrogen content in field collected *P. palmata* rose up to 5.69 and 4% DW, respectively. We observed lowest nitrogen content in *P. palmata* grown at 12 ± 1 °C compared to specimens grown at 15 ± 1 °C and 17 ± 1 °C. This observation could also result from the higher densities in the 12 ± 1 °C the treatment. The determination of total nitrogen using the Kjeldahl method requires no prior extraction of the material, making it a simple, inexpensive, and reproducible procedure (Angell et al. 2015). However, the conversion factor used to calculate protein content with this method is a limitation. The general conversion factor (6.25) assumes that the value of total protein contains 16% nitrogen and that all nitrogen is in the form of protein (Angell et al. 2015). This conversion factor (CF) has been shown to potentially overestimate protein content up to 43%, due to the high content of non-protein nitrogen components in seaweed, such as chlorophylls, nucleic acids, free amino acids and inorganic nitrogen (e.g., nitrate, nitrite and ammonia) (Lourenço et al. 2004). As a result, this can lead to an overestimation of the protein content in seaweed. Consequently, the CF determined by Biancarosa et al. (2017) for *P. palmata* (CF of 4.10), was used for protein content estimation in this study.

While we observed an average protein content of 12 ± 1% DW, Morgan et al. (1980) described considerable variation in crude protein content in both field-growth (11.25–29.38% DW, determined using the 6.25 CF) and tank-grown *P. palmata* material (8.10–23.40% DW). Campos et al. (2022) report an average protein content of 14.40 ± 0.87% DW. *P. palmata* has frequently been noted for its high protein content (Galland-Irmouli et al. 1999; Maehre et al. 2014), commonly as high as 20% and sometimes noted to be up to 35% (Fleurence 1999; Morgan et al. 1980), though the source of this higher estimate is uncertain. Combining the maximum observed total nitrogen content (i.e., 5.69% DW), reported by Chaumont (1978), with the general CF of 6.25, indeed suggests a maximum protein content of 35.6% DW in *P. palmata*. However, using the more accurate CF of 4.10 for *P. palmata*, estimated protein content would be reduced to 23.3% DW. In comparison, protein content in breast muscles in male pheasant and broiler chickens was determined using the Kjeldahl method to be on average 94 and 87% of the dry matter, respectively (Straková et al. 2011), which is 7 to 8 times higher than the average protein content obtained in this study. Kjeldahl analyses indicate that pea seeds (*Pisum sativum* L.) contain 3.5–4.5% nitrogen on a dry matter basis, corresponding to a protein content of 22–25% (Faligowska et al. 2022; Larmure and Munier-Jolain 2019), values comparable to the highest protein levels reported for *P. palmata* in the literature. The relative digestibility of the water-soluble proteins of *P. palmata* to human digestive enzymes, pepsin and pancreatin, was estimated to be 56% (Galland-Irmouli et al. 1999). Unfortunately, seaweed proteins are not fully bioavailable due to the presence of polysaccharides, which can limit their digestibility. For example, strong covalent bonding between mix-linked xylans and glycoproteins complexes, has been reported for *P. palmata* (Dumay et al. 2013; Pliego-Cortés et al. 2020).

Nutrient supply and temperature exhibited a significant effect on the protein-nitrogen content of *P. palmata*. Protein-nitrogen content was significantly higher at 0.5 F/2 medium (regardless of irradiance treatment) and at the more extreme temperatures (15 ± 1 °C and 17 ± 1 °C) compared to the 12 °C treatment. This observation should be interpreted with caution, as the lower nitrogen content might also result from the increased densities in the 12 °C treatment.

In the study by Galland-Irmouli et al. (1999), a seasonal variation of the protein content of field-grown *P. palmata* specimens was observed, with maximum values (21.9 ± 3.5%) in the winter-spring period, when seawater is particularly rich in nutrients, and lower levels (11.9 ± 2.0%) in the summer-early autumn period. The higher protein content observed during the winter-spring season supports the finding that protein-nitrogen content tends to be higher at lower temperatures, although this is more likely to be the result of higher nutrient availability in autumn to spring, in contrast to lower nutrient concentrations over summer. Furthermore, a significant effect of nutrient supply on nitrogen-protein content of *P. palmata* grown at 12 ± 1 °C was observed in this study, regardless of irradiance. Supplying of half of the required nutrients and an irradiance of 19 µmol m^{-2} s^{-1} at 12 ± 1 °C produced the highest protein-nitrogen content. This would be an interesting fact for the cultivation of *P. palmata*, where less nutrients could be used for high protein production. However, this observation should be interpreted with caution, as discussed above. Individuals grown in medium in which half of the F/2 nutrients was administered, may have been primed to nutrient limitation, which might have been only experienced at a later stage by individuals grown in full F/2 medium. Moreover, the finding also contradicts Galland-Irmouli et al. (1999) stating that maximum values for protein content were found when maximum nutrients were available. Morgan et al. (1980) also claim that nitrogen content

(and thus protein-nitrogen content) in tank culture decreases rapidly when *P. palmata* is grown in nitrogen-poor water. For agarophytes and carrageenophytes, it has been established that protein content is dependent on tissue nitrogen status and nitrogen availability in the growth medium (Roleda and Hurd 2019). For example, excess nitrogen is stored in pigments, as was observed in *Gracilaria foliifera* and *Ulva fasciata* (Lapointe 1981; Lapointe and Tenore 1981), causing a decrease in soluble protein content. For seaweeds that have a limited storage capacity, such as *Gracilaria gracilis*, nitrogen pulsing twice a week, produced twice as much yield, protein and pigment contents, rather than thalli pulsed with nitrogen only once a week (Smit et al. 1996).

Carbohydrate Content of P. palmata

The monosaccharide composition of *P. palmata* after acid hydrolysis consists of three monomers, i.e., xylose, galactose and glucose. The sum of the monosaccharide residues was considered to be equal to total sugar content. The total sugar content determined in this study, ranging between 36 and 60% DW (average of 47 ± 5% DW), is similar to the sugar content ranging between 42 and 63% DW, reported by Rødde et al. (2005), in field collected *P. palmata* in the Trondheimsfjord, Norway (April 1998 to September 1999). Campos et al. (2022) reported a carbohydrate content of 34.0 ± 2.2% DW in dulse, available in the Portuguese market for food consumption.

The carbohydrates are mostly composed by xylose (19.4–33.7% DW), galactose (6.70–19.0% DW) and glucose (4.20–13.7% DW). In comparison, Rødde et al. (2005) reported xylose, galactose and glucose levels of 26.9–37.9% DW, 2.9–16.1% DW, and 4.2–18.3% DW, respectively, in in situ collected dulse specimens. It is important to note that the identification of monosaccharides in *P. palmata* suggests the presence of certain polysaccharides, but additional analyses are needed to confirm the polysaccharide composition in terms of linkages and presence of sulphate groups. The major polysaccharide in *P. palmata* is a water soluble β-(1→3) and β-(1→4) linked xylan (Barry et al. 1954; Percival et al. 1950), justifying the presence of xylose. This polysaccharide is unlike the xylans of land plants, which are usually water insoluble and possess only β-(1→4)-linked residues (Morgan et al. 1980). Red algae such as *Palmaria* contain the glycerol glycoside floridoside (2-α-*O*-D-galactopyranosyl glycerol) (Chaumont 1978; Colin and Guéguen 1930), although not quantified in this study, which possibly justifies the presence of galactose residues. Floridoside is known to possess several antifouling and therapeutic properties (Martinez-Garcia et al. 2016) and was found to vary between 3.3 and 25% on a dry weight basis in *P. palmata*, sampled throughout different seasons in the study (Rødde et al. 2005). The glucose residues determined could be possibly part of the floridean starch (amylopectin-like glucan), also characteristic of red algae. Minor amounts of cellulose were also found as structural carbohydrates and can also contribute for the glucose content (Stévant et al. 2023).

Temperature affected sugar composition and content of *P. palmata* grown on the required nutrients. Interestingly, the xylose content was significantly higher at 15 ± 1 °C and 17 ± 1 °C than at 12 ± 1 °C, while for galactose content the opposite trend was observed, regardless of irradiance. This result suggests a promotion of xylan production with temperature. Regardless of irradiance, total carbohydrate content was significantly higher at 17 ± 1 °C compared to 15 ± 1 °C, but not to 12 ± 1 °C. Contrary to these findings, previous studies confirm that higher temperatures have a positive effect on the carbohydrate content of *Ulva* species (He et al. 2018; Wang et al. 2007).

Lipid and Fatty Acid Content of P. palmata

In this work, an average percentage of total lipid content of 0.9 ± 0.2% DW using the B&D protocol was found, similar to what can be found in literature, e.g., 0.3–3.8% DW (Lopes et al. 2019). However, using GC-MS, we obtained an average total fatty acid content of about 0.2 ± 0.1% DW, with a maximum fatty acid content of 0.5% DW. Triacylglycerols (TAGs), also known as triglycerides, are the most common form of lipid storage, consisting of a glycerol molecule esterified with three fatty acids. As the B&D method is a colorimetric technique, it is susceptible to bias from co-extracted non-lipid contaminants (possibly reducing short carbohydrates soluble in chloroform/methanol), which may explain the discrepancy in both values. Therefore, we will focus solely on the fatty acid content in the remainder of the discussion.

In our study, total fatty acid content averaged 2 ± 1 mg g^{-1} DW (0.2 ± 0.1% DW) across all treatments, which is lower compared to the values reported in literature (2.8 ± 0.1% DW, Foseid et al. 2020; 1.1 ± 0.1 and 1.6 ± 0.1%, Schmid et al. 2014). The presence of caprylic acid, capric acid, lauric acid, myristic acid, pentadecylic acid, pentadecenoic acid, palmitic acid, palmitoleic acid, stearic acid, cis-9 oleate, LNA, gamma-linolenate, ALA, arachidonic acid, and EPA was confirmed in *P. palmata* by GC-MS analysis. Similar to the fatty acid composition determined in the current study, Van Ginneken et al. (2011) reported that EPA and palmitic acid accounted for 59% and 25% of the total fatty acid content, respectively. Lopes et al. (2019) confirmed the dominant prevalence of EPA and palmitic acid in *P. palmata* with a relative abundance of 51.7 ± 6.5% and >13.2%, respectively. Schmid et al. (2014) also identified *P. palmata* to be the most promising species as a source of 20:5 n-3

(EPA) amongst 16 species collected from the Irish west Coast, with levels ranging from 36.8±4.3 to 41.2±1.8% of total FA content in November and June, respectively. Seaweed generally contains higher levels of polyunsaturated fatty acids, particularly omega-3 fatty acids, while red meat for example tends to have higher levels of saturated fats and monounsaturated fats (Rocha et al. 2021; Williamson et al. 2005). For example, EPA content in loins and dry cured ham from pigs was less than 1% of total FA content, while saturated fatty acid content was above 30% of total FA content in pig flesh (Vossen et al. 2017). Considering a moisture content of 71%, EPA content in loins from soy fed pigs was on average 0.03 mg g^{-1} DW (Vossen et al. 2017), which is considerably lower than the EPA content we recovered in *P. palmata* in all treatments (0.8±0.7 mg g^{-1} DW, regardless of treatment), despite a lower total fatty acid content present in the seaweed. In contrast, pea fatty acids are predominantly palmitic, linoleic and linolenic acid, with no detectable levels of DHA or EPA (e.g., Ciurescu et al. 2018). The EFSA Panel on Dietetic Products, Nutrition, and Allergies (2010) recommends a daily intake of 250 mg EPA+DHA per day for adults to maintain their overall health. Together, this suggests that while *P. palmata* can be a useful addition to meet the recommended omega-3 fatty acid needs, it is unlikely to be the sole provider in a human diet due to its low content of these fatty acids.

Temperature significantly influenced fatty acid composition of *P. palmata* (effect on caprylic acid, myristic acid, palmitic acid, palmitoleic acid, stearic acid, cis-9 oleate, LNA content and sum of FAs, regardless of irradiance; Table S3). Fatty acid concentrations were significantly higher at a lower temperature (12±1 °C), regardless of irradiance treatment. Remarkably very few studies have investigated changes in the fatty acid profile of seaweed species reared under different temperature conditions. One notable exception is the study by Toth et al. (2020) who observed exposure to higher temperatures resulted in higher FA levels in cultivated *Ulva fenestrata* (Chlorophyta), in contrast to our findings. Most studies report (seasonal changes in) the FA profile in wild harvested seaweed biomass, making determination of the responsible environmental driver less straightforward (e.g., Osuna-Ruiz et al. 2019; Paiva et al. 2018; Pereira et al. 2021; Schmid et al. 2014). For *P. palmata*, the total fatty acid contents decreased from 1.6 to 1.1%, comparing content in harvests in November and June, respectively (Schmid et al. 2014). Samples collected from several species in Egypt (3 species, i.e., *Ulva lactuca*, *Jania rubens*, and *Pterocladia capillacea*) and Ireland (14 species, among which *P. palmata*) presented variable FA concentrations in spring and summer compared to autumn (Khairy et al. 2013; Schmid et al. 2014). Limited available research suggest however that the FA profile can be linked to the species and not just taxonomic groups, yielding distinct FA compositions and seasonal variabilities (Schmid et al. 2014).

UFAs are considered to be anti-atherogenic as they inhibit the accumulation of plaque and reduce the levels of phospholipids, cholesterol, and esterified fatty acids (Chen and Liu 2020). Therefore, the consumption of fatty foods or products with a lower AI can reduce the levels of total cholesterol and LDL-C in human blood plasma. Whether this is also the case for food sources with an overall low FA content, such as is the case in seaweed, needs to be further investigated. Here, we report AI values ranging from 1.2 to 17.7 with significantly lower AI values in *P. palmata* grown at 12±1 °C (AI<1.9), versus higher temperatures (regardless of irradiance treatments). The AI has been used widely for evaluating seaweeds, crops, meat, fish, dairy products, etc. For example, AI was found to range from 4.08 to 5.13 in milk from cows at different stages of lactation (Nantapo et al. 2014). For seaweeds, the species itself may also influence the AI value, as a range from 0.03 to 3.58 was summarized in Chen and Liu (2020) for 41 species, including 17 species of Rhodophyta. The TI characterises the thrombogenic potential of FAs, assessing their propensity to cause blood clot formation in vessels. Here we report TI values ranging from 0.2 to 6.1, also with a temperature dependent effect (Fig. 8), with TI values lower than 0.44 for *P. palmata* grown at 12±1 °C. For seaweeds in general, the value was found to range from 0.04 to 2.94 except for *Gracilaria salicornia*, which had a TI value of 5.75 (Chen and Liu 2020; Kumar et al. 2010). Chen and Liu (2020) also summarised ranges of TI values for crops, fish, meat, and dairy products, being 0.139–0.56, 0.14–0.87, 0.288–1.694, and 0.39–5.04, respectively. In terms of human health, AI and TI values less than 1.0 and 0.5, respectively, in the diet, are recommended (Fernandes et al. 2014). Our results suggest that, if reared under certain conditions, lipid content of *P. palmata* is of good quality and indicate a higher pro-health value. Environmental conditions consisting of an irradiance intensity of 14 µmol m^{-2} s^{-1}, a temperature of 12 °C and optimal nutrient availability showed minimal AI and TI levels, which also coincided with the highest growth rate observed.

R-Phycoerythrin Content of P. palmata

Gallagher et al. (2021) reported a concentration of R-phycoerythrin of 6.078 mg g^{-1} DW in screw-pressed dry material of wild-growing *P. palmata*, collected in Swansea, Wales in June (extracted with 0.1 M of potassium phosphate buffer, pH 6.5), which is extensively higher than what is reported in this study (average of 657±201 µg g^{-1} DW, regardless of treatment). Similarly, Dumay et al. (2013) reported R-phycoerythrin recovery rates in *P. palmata* ranging from 110 to 1150 µg g^{-1} DW depending

on the applied extraction process. In general, both macro- and micro-algal cells tend to enhance pigment synthesis to harvest available photons at lower light intensities (e.g., Ak and Yücesan 2012; Chaloub et al. 2015; Lapointe 1981; Xie et al. 2021). For example, total chlorophyll, R-phycoerythrin and R-phycocyanin were highest in the *Gracilaria verrucosa* when reared at 25 µmol m^{-2} s^{-1}, compared to 50, 75, 100 and 150 µmol photons m^{-2} s^{-1}. Similarly, R-phycoerythrin content decreased with almost 40% after exposure to 500 µmol photons m^{-2} s^{-1} compared to specimens acclimated to 40 µmol m^{-2} s^{-1} (22.05 to 13.96 mg g^{-1} DW; Carnicas et al. 1999). Lapointe (1981) demonstrated that reduced pigment content did not affect the growth capacity of *Gracilaria foliifera*. However, in this work, R-phycoerythrin levels were not significantly higher at an irradiance of 14 µmol m^{-2} s^{-1} compared with 19 µmol m^{-2} s^{-1} (regardless of temperature treatment), which indicates that the difference in light intensity was probably too small to possibly the observation of any significant changes in the seaweed' composition.

Nevertheless, we did find a temperature dependent effect in R-phycoerythrin composition, with higher pigment contents in dulse reared at 12 ± 1 °C compared to the higher temperatures (regardless of irradiance), although we cannot exclude density-related impacts as well (e.g., through self-shading, due to the higher stocking densities). Mizuta et al. (2002) observed high R-phycoerythrin levels in red macroalgae *Gloiopeltis furcata* and *Porphyra yezoensis*, followed by a decrease in late spring or summer, with accompanying discoloration from deep red to green or yellow, corresponding to changes in the water temperature and nutrient availability. These findings are in sharp contrast to the findings of Corey et al. (2012a) who noted the R-phycoerythrin content of *P. palmata* being independent of temperature (exposure to 6, 10, or 17 ± 1 °C for 4 weeks). The authors did find a significant nutrient dependence effect, as well as an interaction effect. Highest average R-phycoerythrin content of 23.6 mg g^{-1} FW^{-1} was found in specimens grown at 17 ± 1 °C in medium with 300 µM nitrate. Several studies report higher R-phycoerythrin content in *P. palmata* and other red macroalgae when the tissue N content is higher as well (e.g., Amano and Noda 1987 ; Corey et al. 2012a; Lapointe 1981, 1985; Mizuta et al. 2002). Despite this positive correlation has been established in these studies, we observed a negative correlation between the two parameters. Yet, observations from the other studies indicate that R-phycoerythrin plays an important role as a nitrogen pool for red algae. It is possible that *P. palmata* may have other mechanisms more important than phycoerythrin for the storage of nitrogen at higher temperatures, as has been suggested for *Chondrus crispus*, for example (Corey et al. 2012a). Moreover, in some of the *P. palmata* fronds growing at 15 ± 1 °C or 17 ± 1 °C, some green discolouration was observed in the week of harvesting. While these macroalgae typically have a distinct red colour, individuals may turn greenish or even bleach due to higher temperatures and increased sunlight exposure during the early summer months, causing changes in pigment composition (Stévant et al. 2023). Similarly, Corey et al. (2012a) observed discoloration in both *P. palmata* and *C. crispus* exposed at low nitrogen availability conditions at the tips of fronds where the frond is thinner, whilst the basal part of these fronds still contained a significant amount of pigment. The authors demonstrated that phycoerythrin accumulates rapidly at high nitrogen concentrations, leading to increased photosynthetic activity and growth. However, we could not demonstrate that nutrient supply significantly affected phycoerythrin content in our study. However, it could be possible, due to the nitrogen-storing properties of phycoerythrin, the seaweed experienced less consequences from a lower nutrient supply, explaining the small number of significant changes in the chemical composition of the seaweed. Finally, the observed negative correlation between phycoerythrin and nitrogen content could indicate lower light availability through self-shading, due to the higher stocking densities observed in the 12 ± 1 °C treatment at 12 weeks.

Antioxidant Activity of P. palmata and Human Health Potential

An important group of seaweeds' primary and secondary metabolites, such as phenolics, polysaccharides, fatty acids, carotenoids and sterols, are described in literature to display antioxidant activity, i.e., the ability to neutralise free radicals by behaving as "free radical scavengers" and as such, preventing or repairing damages caused by oxidative stress (Liu and Sun 2020; Meenakshi et al. 2011). Here, we observed an average antioxidant content of 0.73 ± 0.16% DW (7.3 ± 1.6 mg ascorbic acid eq g^{-1} DW) in *P. palmata* across all treatments. This antioxidant content encompasses vitamin E, carotenoids, flavonoids and cinnamic acid derivatives. In comparison, Meenakshi et al. (2011) determined antioxidant content in *Sargassum wightii* to be 123.40 ± 4.00 mg ascorbic acid eq g^{-1} DW using the same assay, which can be attributed to higher phenolic content compared to green and red macroalgae.

Antioxidants are significantly impacted by light stress, as most reactive oxygen species (ROS) are byproducts during photosynthesis, especially when saturated at higher irradiances (Foyer 2018). As expected, due to the low light irradiances selected to grow *P. palmata*, we did not observe any significant differences in antioxidant content among the different irradiance treatments (regardless of temperature). Temperature had a significant effect on antioxidant activity in *P. palmata* specimens with a significantly lower antioxidant

content in the specimens grown at 15 ± 1 °C and 17 ± 1 °C, compared to 12 ± 1 °C (regardless of irradiance). The applied light intensities and the low nutrient levels did not appear to act as stress triggers, as no significant effects were induced. Suboptimal conditions, such as excessive temperatures, or stress-induced damage increase the production of ROS molecules in seaweed (e.g., Bischof and Rautenberger 2012). Our results disproved the expectation that higher temperatures would lead to increased production of antioxidants to neutralise ROS molecules. However, it could be possible that ROS production only occurs in the initial phase after exposure to heat stress, as observed by Kumar and collaborators (2020). The authors observed peak production of ROS occurred in *Kappaphycus alvarezii* (tropical red macroalga) within the first hour after exposure to elevated temperature, before decreasing. Their results suggest that the antioxidant activity occurred mainly during the initial phase of the temperature change and that the seaweeds subsequently activated other defence mechanisms to cope with the ongoing thermal stress.

Conclusion

Changes in biochemical composition of macroalgae in response to various environmental conditions is a complex process that is not yet fully understood. Therefore, this study explored suboptimal conditions for cultivating *P. palmata*, aiming to harmonise biomass growth with chemical composition. We observed highest growth rates for *P. palmata* after cultivation in water of 12 ± 1 °C, as higher temperatures significantly diminish growth rates, while a small change in low light conditions (14 versus 19 µmol m^{-2} s^{-1}) did not impact growth rate significantly. To maximise the health benefits of seaweed as food, a balance must be struck between growth and chemical composition of the seaweed. A comprehensive analysis was carried out to evaluate the nutritional value and identify several bioactive compounds in *P. palmata* grown under different environmental conditions. Carbohydrate content and profile were influenced by temperature, with a positive effect of increased temperature on the xylan content, while lower temperature promoted the production of galactose containing compounds, possibly floridoside. Moreover, both total antioxidant activity and R-phycoerythrin content reached their highest levels at the lowest water temperature tested. Higher temperatures also significantly altered fatty acid composition, increasing AI and TI indices, which indicates a lower lipid quality. Continued research efforts can be directed towards exploring the potential for large-scale and sustainable aquaculture of *P. palmata* under optimal culture conditions, with the aim of using this seaweed as a widespread and nutritious food source.

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Data Availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Conflict of Interest The authors declare that they have no conflict of interest.

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