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Effects of low-temperature stress on the green and brown strains of *Kappaphycus alvarezii*

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ABSTRACT

The present study aimed to evaluate the effects of thermal variation on the green and brown strains of *Kappaphycus alvarezii* cultivated under laboratory conditions. The seaweeds were maintained in two cultivation regimes for 42 days: a control group at 26 ± 0.5 °C and an experimental group subjected to a gradual decrease from 24 ± 0.5 °C to 14 ± 0.5 °C, with a 2 °C reduction per week. Growth was monitored weekly and documented with photographic records. Biomass samples were collected at 22, 18, and 14 ± 0.5 °C for analyses of photosynthetic pigments and phenolic compounds, while morphological evaluations using optical microscopy were carried out after exposure to 18 and 14 ± 0.5 °C. The gradual decrease in temperature impaired daily growth in both strains, with a pronounced reduction from 18 ± 0.5 °C and minimum values at 14 ± 0.5 °C. The green strain maintained greater growth stability, while the brown strain preserved photosynthetic pigments more effectively under control conditions and exhibited higher levels of phenolic compounds at low temperatures. In addition, significant interactions between strain and treatment were observed for phycobiliproteins (phycoerythrin and phycocyanin), indicating that the response to cold stress results from the combination of these factors. These findings highlight that the green and brown strains exhibited distinct

physiological, morphological, and biochemical responses to thermal stress, reinforcing the importance of selecting cold-tolerant strains to optimize productivity and mitigate seasonal risks in regions with harsh winters.

Keywords: Algiculture, red algae, thermal stress, growth, photosynthetic pigments, phenolics.

1 INTRODUCTION

Aquaculture has consolidated itself as one of the most dynamic sectors in food production, job creation, and global economic development (FAO. 2020). In addition to the cultivation of aquatic organisms, seaweed production has shown remarkable growth, reaching 37 million tons in 2022, with a market value estimated at 17 billion dollars (FAO. 2025). Macroalgae, multicellular photosynthetic organisms, are recognized not only for their nutritional value but also for their broad industrial applicability, encompassing the food, pharmaceutical, agricultural, and biotechnology sectors (Zhang et al. 2022; Wu et al. 2023; Kamble et al. 2025; Balkrishna et al. 2025).

Among the cultivated species, *Kappaphycus alvarezii* (Doty) Doty ex Silva (Rhodophyta, Gigartinales) stands out as one of the most widely farmed seaweeds worldwide, currently ranking sixth in commercial importance (FAO. 2025; Hurtado, 2022). This species exhibits a high capacity for biomass production in short periods, being able to double its size every 15 to 30 days (Hinaloc and Roleda 2021). Its wide variation in coloration, attributed to pigments such as chlorophylls, carotenoids, and phycobiliproteins, together with its nutritional composition and high carrageenan content, reinforces its value across different production chains (Ranganayaki et al. 2014; Hinaloc and Roleda 2021; Mamat et al. 2024). The species is typical of tropical waters of the Pacific and Indian Oceans, where different morphotypes, haplotypes, and color strains such as green and brown have already been described (Zuccarello et al. 2006; Conklin et al. 2009; Tan et al. 2013).

In Brazil, K. alvarezii was introduced in 1995, with green and brown strains derived from genetic selection programs conducted in Malaysia and the Philippines (Paula et al. 1999; Instituto de Pesca 2023), and its cultivation is currently consolidated in the state of Santa Catarina, Southern Brazil. The 2023/2024 harvest reached 751 tons, generating revenues of over BRL 10 million (≈ USD 1.9 million), with a large portion of the biomass destined for the production of seaweed liquid extract (Santos 2024). This

extract, rich in phytohormones and nutrients, has demonstrated effectiveness as a plant biostimulant in various crops (Gelli and Barbieri 2015; Nunes et al. 2024; Hermans 2024).

Despite this promising scenario, the cultivation of *K. alvarezii* in southern Brazil still faces seasonal challenges, mainly during winter. During this season, water temperature can drop below 18 °C, leading to high mortality rates associated with the "ice-ice" disease (Hayashi et al. 2011; Alibon et al. 2019). This condition is characterized by pigment loss and thallus softening and has been linked to both pathogenic bacteria and to abiotic stresses that favor its onset (Largo et al. 1995; Achmad et al. 2016; Azizi et al. 2018).

Studies such as those by Li et al. (2016) demonstrated that *K. alvarezii* has moderate cold tolerance, activating antioxidant mechanisms and maintaining photosynthesis between 20 °C and 23 °C. However, at 14 °C, photosynthetic efficiency decreases, and the antioxidant response fails, indicating that this temperature represents a critical physiological limit for the species (Li et al. 2016b). Furthermore, Mandal et al. (2015) observed that temperatures between 20 °C and 30 °C favor growth and fragment regeneration, whereas values outside this range compromise nutrient uptake and thallus integrity. Consistently, Hayashi et al. (2011) reported better performance during summer in the waters of Southern Brazil, with average temperatures ranging from 27.1 °C to 27.9 °C, reinforcing that *K. alvarezii* achieves its highest productivity in tropical environments, particularly within the 20 °C to 32 °C range.

Beyond its effects on growth, temperature also influences the phenotypic diversity of the species. Gelli et al. (2024) demonstrated that averages around 22.88 ± 1.8 °C favored the natural appearance of different coloration patterns, whereas lower values reduced this occurrence. These findings indicate that temperature affects not only productivity but also the phenotypic diversity observed in cultivated strains.

Although numerous studies have addressed the effects of high temperatures on seaweeds (Kumar et al. 2020; Keng et al. 2021; Syahrul et al. 2023), responses to low temperatures have been less explored, particularly in commercially important strains of *K. alvarezii*. In this context, understanding the physiological and morphological responses of green and brown strains is of paramount importance for the sustainability and expansion of seaweed farming in Southern Brazil, directly aligned with the Sustainable Development Goals (SDGs). The present study evaluated the effects of gradual temperature decrease on growth, photosynthetic pigments, phenolic compounds, and morphology of the green and brown strains of *K. alvarezii*. These insights aim to

guide the selection of more tolerant genotypes and contribute to strengthening seaweed farming in regions subject to harsh winters.

2 MATERIALS AND METHODS

2.1 Cultivation conditions

Two *K. alvarezii* strains, green and brown, previously available at the Macroalgae Sector of the Marine Shrimp Laboratory (LCM), of the Federal University of Santa Catarina (UFSC), were used. The strains underwent an initial 30-day growth period to obtain the acclimatized biomass, using sterilized seawater with a salinity of 35 ± 1 ‰, temperature of 26 ± 1 °C, a 12 h photoperiod, irradiance of 100 ± 10 µmol photons m^{-2} s^{-1}, and constant aeration.

2.2 Experimental design

This study evaluated the effects of gradual temperature reduction on daily growth, phenolic compounds, photosynthetic pigments, and morphology of green and brown strains of *K. alvarezii* under laboratory conditions for 42 days. Each strain was represented by 24 g of fresh biomass, subdivided into units containing 1 g of thalli in 250 mL of sterilized seawater supplemented with 50% von Stosch nutrient solution, renewed weekly. Cultures were maintained under a 12 h photoperiod, irradiance of 100 ± 10 µmol photons m^{-2} s^{-1}, and constant aeration.

Two thermal conditions were tested: (a) control, maintained at 26 ± 0.5 °C, and (b) gradual reduction, starting at 24 °C and decreased by 2 °C per week until 14 °C. A chiller system (Gelaqua) ensured 7 days of exposure at each temperature (24, 22, 20, 18, 16, and 14 ± 0.5 °C). Each treatment had three independent biological replicates per strain, represented by separate culture units. Growth was monitored weekly by biomass measurements. Water conditions remained stable, with mean salinity of 33.17 ± 0.21 ‰ and pH of 7.91 ± 0.07.

Samples for total phenolics and pigment analyses were collected after 7 days at 22, 18, and 14 ± 0.5 °C, with controls at 26 ± 0.5 °C. Morphological analyses were conducted after 7 days of exposure to 18 and 14 ± 0.5 °C and under control conditions.

These sampling points allowed thalli to acclimate before analyses, with temperatures reflecting local winter records provided by the Agricultural Research and Rural Extension Company of Santa Catarina (EPAGRI, Brazil; personal communication).

2.3 Assessment of daily growth rate of macroalgae

Over six weeks, seaweed growth was monitored by weekly weighing on an analytical balance. Thalli were gently blotted with absorbent paper before determining fresh biomass. The daily growth rate (DGR, % day^{-1}) was calculated separately for each week, using the formula adapted from Yong et al. (2013):

$$DGR = \left(\left(B_f/B_i\right)^{(1/t)} - 1\right) * 100$$

Where *Bf* is the biomass in the current week (g), *Bi* the biomass in the previous week (g), and *t* the number of days in the interval ($t = 7$).

2.4 Cortical cell wall thickness

Samples of approximately 5 mm in length were collected at 18 °C and 14 °C and processed for light microscopy analysis, following the protocol adapted from (Bouzon 1993). Samples were initially fixed in 2.5% paraformaldehyde solution prepared in 0.1 M sodium phosphate buffer (pH 7.2), dehydrated in a graded ethanol series, and embedded in glycol methacrylate historesin for polymerization. The resulting blocks were sectioned into 4 μm transverse sections using a microtome (Leica RM 2125), stained with toluidine blue, and analyzed under a light microscope (Olympus BX41, LAMEB/CCB/UFSC) equipped with an epifluorescence system, using a 40× objective.

The thickness of the external cell wall was determined from histological images using ImageJ® software. Measurements were performed on cortical cells with the Straight-Line Tool, after calibration of the scale present in each image. For each treatment, 15 measurements were obtained from three independent sections, totaling 45 measurements per group. Results were expressed as mean values ± standard deviation (SD).

2.5 Photosynthetic pigment analyses

2.5.1 Chlorophyll *a* and Carotenoids

The quantification of chlorophyll *a* and carotenoids was performed according to Wellburn (1994). For this, 0.4 g of sample from each treatment was used. Pigments were extracted with 1 mL of dimethyl sulfoxide (DMSO, Merck, Germany) at 40 °C for 40 minutes. Absorbance readings were taken with a spectrophotometer (Infinite M200 microplate reader, TECAN) at wavelengths of 480, 649, and 665 nm. Chlorophyll *a* and carotenoid concentrations were determined using the following equations:

$$Chlorophyll\ a = (12.19 \times A_{665}) - (3.45 \times A_{649})$$

$$Carotenoids = \frac{(1000 \times A_{480}) - (2.14 \times Chlorophylla)}{220}$$

The analyses were carried out in triplicate, and the results were expressed as μg of pigments per g of fresh weight.

2.5.2 Phycobiliproteins

The extraction and quantification of phycobiliproteins (phycoerythrin, phycocyanin, and allophycocyanin) were performed according to Kursar et al. (1983). For this, 0.3 g of biomass were homogenized using an Ultra-Turrax Dremel 3000. The suspension was centrifuged for 10 min at approximately 472 × g and 4 °C, and the supernatant was collected and kept in the dark until absorbance measurements (Infinite M200 Multireader, TECAN) at 498, 614, and 651 nm. Phycobiliprotein concentrations were calculated using the following equations:

$$\text{Phycoerythrin} = (155.8 \times A_{498}) - (40 \times A_{614}) - (10.5 \times A_{651})$$

$$\text{Phycocyanin} = (151.1 \times A_{614}) - (99.1 \times A_{651})$$

$$\text{Allophycocyanin} = (181.3 \times A_{651}) - (22.3 \times A_{614})$$

The analyses were carried out in triplicate, and the results were expressed as μg of pigments per g of fresh weight.

2.6 Extraction of total phenolic compounds

The quantification of total phenolics was performed according to Schiavon et al. (2012), with modifications. Methanolic extracts were prepared from 500 mg of dry

biomass, previously ground in liquid nitrogen and extracted with 5 mL of 80% methanol, and kept in the dark for 1 h. Samples were then centrifuged at 153.8 × g for 7 min, and the supernatant was collected. For the determination of total phenolic compounds, 300 µL of the extract were mixed with 225 µL of Folin–Ciocalteu reagent and 2.475 mL of 2% sodium carbonate solution. The mixture was vortexed and incubated in the dark for 1 h. Subsequently, aliquots of 300 µL were transferred to microplates, in triplicate, and absorbance was measured at 750 nm using a spectrophotometer.

2.7 Statistical analysis

Homoscedasticity and normality of all data were assessed using Bartlett's and Shapiro–Wilk tests, respectively. Data were then subjected to factorial analysis of variance (ANOVA) (Strain × Treatment), followed by Tukey's test, and when assumptions were not met, to the Kruskal–Wallis test. All statistical analyses were performed in Statistica 13.5 (TIBCO Software Inc.), with a significance level of 5% ($p < 0.05$). Results were expressed as mean values ± standard deviation (SD).

3 RESULTS

3.1 Daily growth rate and thallus morphology

In week 1, when the thermal variation samples were at 24 °C, significant differences were observed between the green control group and the brown control ($p = 0.0267$), green under thermal variation ($p = 0.0321$), and brown under thermal variation ($p = 0.0035$). The mean growth rates were 9.49% day^{-1} in the green control, 5.08% day^{-1} in the brown control, 5.24% day^{-1} in the green under thermal variation, and 3.16% day^{-1} in the brown under thermal variation (Fig. 1).

In weeks 2 and 3, when the thermal variation samples were at 22 °C and 20 °C, respectively, no significant differences were detected among the groups. In week 2, mean daily growth rates were 9.15% day^{-1} in the green control, 5.06% day^{-1} in the brown control, 10.12% day^{-1} in the green under thermal variation, and 4.77% day^{-1} in the brown under thermal variation (Fig. 1). In week 3, mean daily growth rates were 5.99% day^{-1} in the green control, 3.08% day^{-1} in the brown control, 3.67% day^{-1} in the green under thermal variation, and 2.00% day^{-1} in the brown under thermal variation (Fig. 1).

In week 4, when the thermal variation samples were at 18 °C, significant differences were observed between the green control and the brown under thermal variation ($p = 0.0358$), as well as between the brown control and the green under thermal variation ($p = 0.0232$) and the brown under thermal variation ($p = 0.0079$). The mean growth rates were 3.49% day^{-1} in the green control, 4.27% day^{-1} in the brown control, 1.64% day^{-1} in the green under thermal variation, and 1.08% day^{-1} in the brown under thermal variation (Fig. 1).

In week 5, when the thermal variation samples were at 16 °C, a significant difference was observed between the brown under thermal variation and the brown control ($p = 0.0279$). The mean growth rates were 3.31% day^{-1} in the green control, 4.72% day^{-1} in the brown control, 0.88% day^{-1} in the green under thermal variation, and 0.36% day^{-1} in the brown under thermal variation (Fig. 1).

In week 6, when the thermal variation samples were at 14 °C, a significant difference was observed between the brown under thermal variation and the green control ($p = 0.0395$). The mean growth rates were 4.51% day^{-1} in the green control, 4.00% day^{-1} in the brown control, 0.34% day^{-1} in the green under thermal variation, and 0.07% day^{-1} in the brown under thermal variation (Fig. 1). The polynomial adjustment of these rates for each treatment and strain is presented in the supplementary information (Fig. S1), allowing visualization of growth trends throughout the experiment under control and thermal variation conditions.

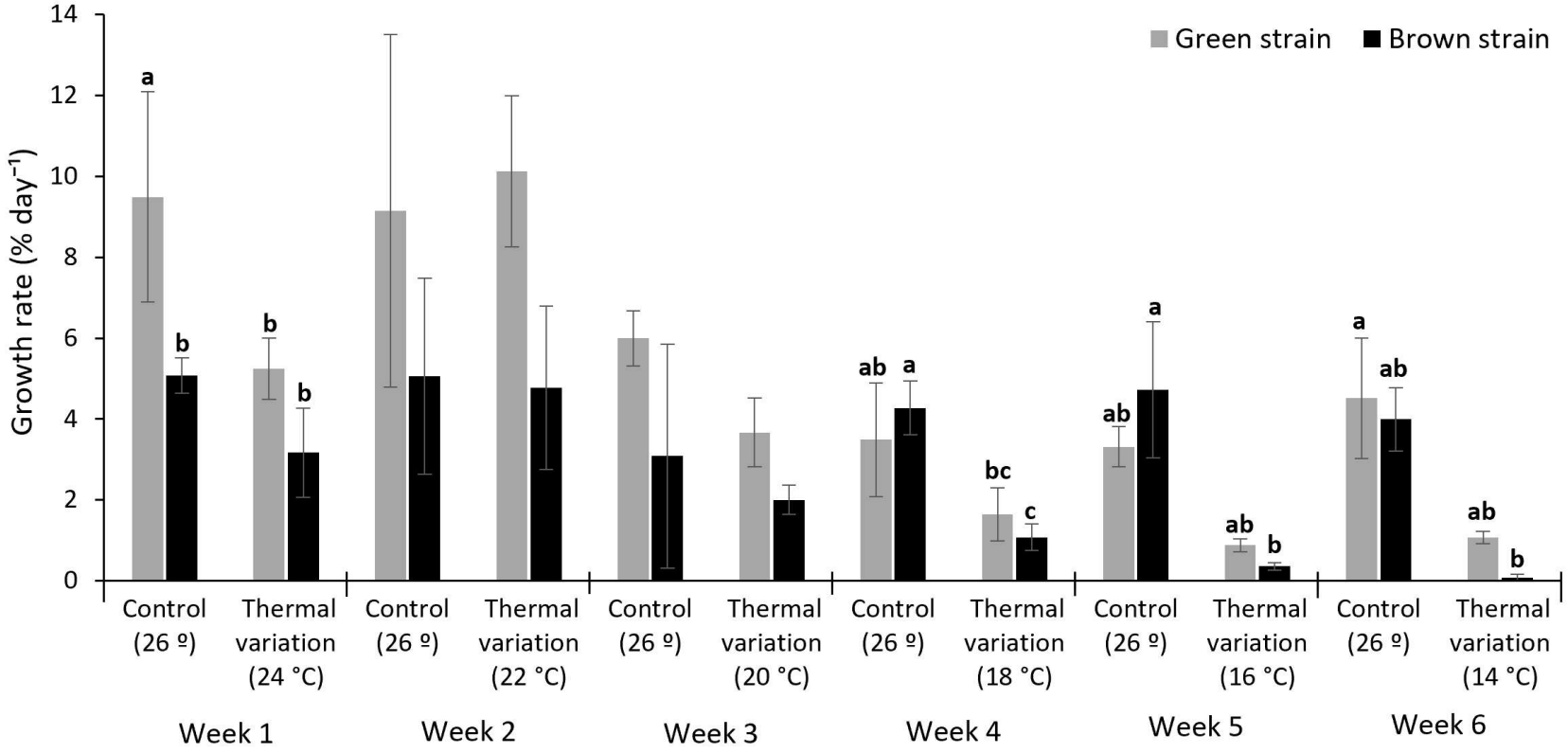


Fig.1. Mean daily growth rate (% day^{-1}) of *Kappaphycus alvarezii* green and brown strains cultivated at 26 °C (control) and under thermal variation, in which seaweed remained for 7 days at each temperature (24 °C, 22 °C, 20 °C, 18 °C, 16 °C, and 14 °C). Bars represent mean ± standard deviation of triplicates. Statistical analyses were performed separately for each week, and different letters (a, b, c, ab, bc) indicate significant differences among groups ($p < 0.05$, Tukey's test).

Morphological changes associated with cultivation at different temperatures are presented in Fig. 2 (photographs). Under thermal variation, the brown strain exhibited pigment loss starting at 16 °C, whereas the green strain retained more intense coloration throughout the experiment, with pigment loss becoming evident only at 14 °C, characterized by translucent apices. Under control conditions (26 °C), both strains maintained structural integrity and pigmentation.



Fig. 2. Morphological aspects of *Kappaphycus alvarezii* thalli cultivated under thermal variation from 24 °C to 14 °C (A, C) and under control conditions at 26 °C (B, D). Images show the green strain (A, B) and the brown strain (C, D). The first row corresponds to thalli standardized with an initial biomass of 1 g at the beginning of the experiment, while the others were obtained after 7 days of cultivation under each condition. The scale bar in the images indicates thallus size.

3.2 Cortical cell wall thickness

In light microscopy, using the histochemical test with toluidine blue, it was possible to identify an intense metachromatic reaction, indicating the presence of acidic polysaccharides in the cell wall (Fig. 3A), consistent with the presence of carrageenan. In addition, a more pronounced thickening of the outer cortical cell wall was evident, mainly under thermal variation conditions, which corroborates the greater flexibility observed in

the thalli of the brown strain, suggesting a relationship with the composition of its cell wall.

The microscopy findings corroborate the statistical results, in which, at sampling 1, when the thermal variation samples were at 18 °C, a significant interaction was observed between strain and treatment factors ($p = 0.0049$). Statistical differences were found between the brown under thermal variation and the brown control ($p = 0.0004$), green control ($p = 0.0001$), and green under thermal variation ($p = 0.0005$). The mean values obtained were 4.92 µm in the green control, 6.28 µm in the brown control, 6.43 µm in the green under thermal variation, and 12.42 µm in the brown under thermal variation (Fig. 3B).

At sampling 2, when the thermal variation samples were at 14 °C, a significant difference was found between the brown under thermal variation and the brown control ($p = 0.0007$), green control ($p < 0.0001$), and green under thermal variation ($p = 0.0062$). In addition, the green control differed from the green under thermal variation ($p = 0.0136$). The mean values obtained were 4.39 µm in the green control, 7.05 µm in the brown control, 9.36 µm in the green under thermal variation, and 15.04 µm in the brown under thermal variation (Fig. 3B).

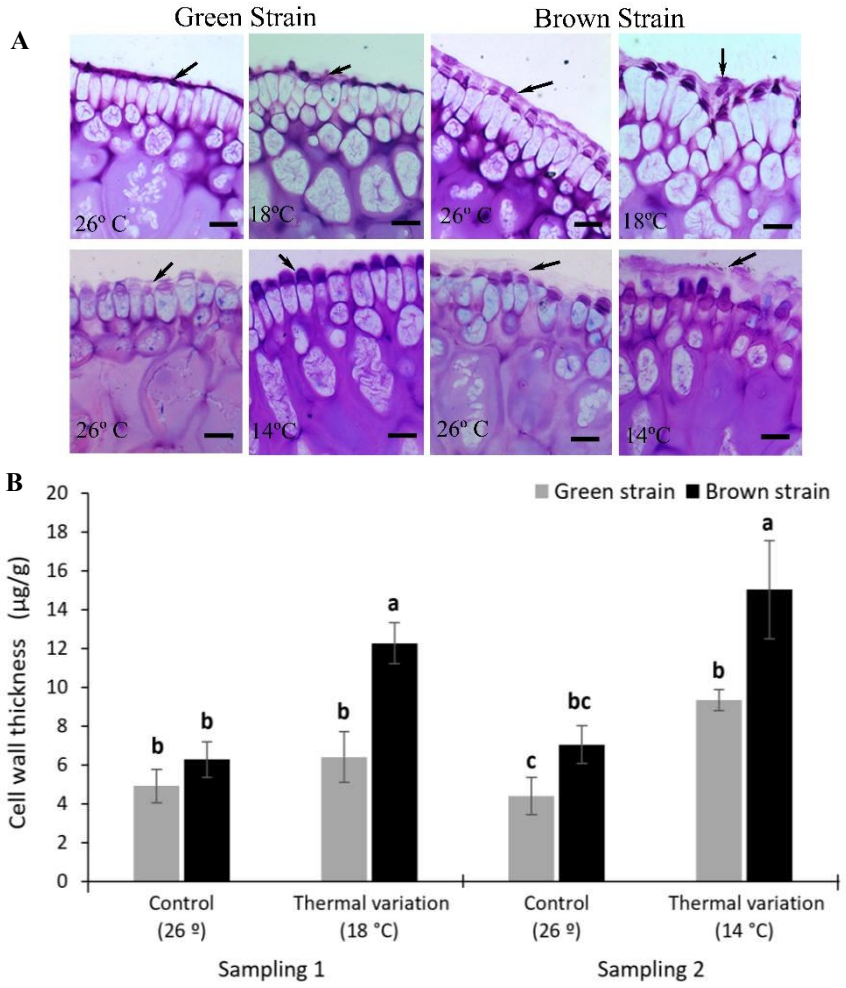


Fig. 3. (A) Transverse sections of thalli from the green and brown strains of *Kappaphycus alvarezii* cultivated under control conditions (26 °C) and thermal variation (18 °C and 14 °C), obtained by light microscopy after staining with toluidine blue. Arrows indicate cell wall thickening. Scale bar = 10 µm. (B) Mean cell wall thickness (µm) under control (26 °C) and thermal variation (18 °C and 14 °C). Bars represent mean ± standard deviation of triplicates. Statistical analyses were performed separately for each sampling, and different letters (a, b, c, bc) indicate significant differences among groups ($p < 0.05$, Tukey's test).

3.3 Photosynthetic Pigment Analyses

3.3.1 Chlorophyll *a*

At sampling 1, when the thermal variation samples were at 22 °C, no significant differences were observed among groups. The mean values were 59.32 µg/g for green control, 62.30 µg/g for brown control, 31.18 µg/g for green under thermal variation, and 29.36 µg/g for brown under thermal variation, as shown in Fig. 4.

At sampling 2, when the thermal variation samples were at 18 °C, no significant differences were observed among groups. The mean values were 106.13 µg/g for green control, 102.87 µg/g for brown control, 137.96 µg/g for the green under thermal variation, and 125.49 µg/g for the brown under thermal variation, as shown in Fig. 4.

At sampling 3, when the thermal variation samples were at 14 °C, a significant difference was found between the brown control group and the green under thermal variation ($p = 0.0039$) and brown under thermal variation ($p = 0.0064$). The mean values obtained were 27.32 µg/g in the green control group, 47.01 µg/g in the brown control, while the lowest concentrations were recorded in the strains under thermal variation, with 9.37 µg/g in the green under thermal variation and 12.38 µg/g in the brown under thermal variation, as shown in Fig. 4.

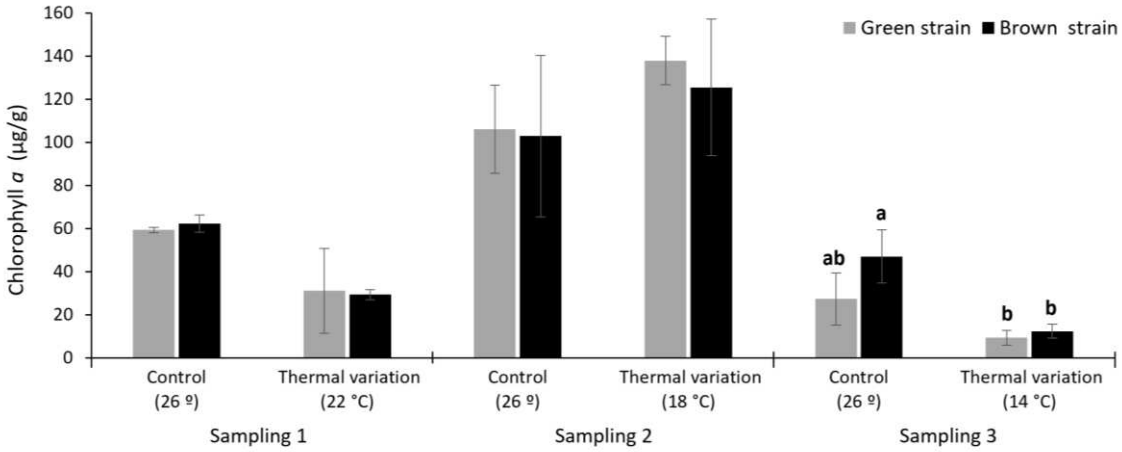


Fig. 4. Chlorophyll *a* level (µg/g) of green and brown strains of *Kappaphycus alvarezii* cultivated at 26 °C (control) and under thermal variation, with samplings performed at 22 °C, 18 °C, and 14 °C. Bars represent mean ± standard deviation of triplicates. Statistical analyses were performed separately for each sampling, and different letters (a, b, ab) indicate significant differences among groups ($p < 0.05$, Tukey's test).

3.3.2 Carotenoids

At sampling 1, when the thermal variation samples were at 22 °C, there was a significant difference between the brown control group and the green control ($p = 0.0072$) and green under thermal variation groups ($p = 0.0015$). The mean values were 4.89 µg/g in the green control group, 10.22 µg/g in the brown control, 3.36 µg/g in the green under thermal variation, and 6.84 µg/g in the brown under thermal variation, as shown in Fig. 5.

At sampling 2, when the thermal variation samples were at 18 °C, there was a significant difference between the brown under thermal variation group and the green control ($p = 0.0183$) and brown control groups ($p = 0.0425$). The mean values were 14.03 µg/g in the green control group, 16.50 µg/g in the brown control, 25.98 µg/g in the green under thermal variation, and 29.88 µg/g in the brown under thermal variation, as shown in Fig. 5.

At sampling 3, when the thermal variation samples were at 14 °C, there was a significant difference between the green control and brown control groups ($p = 0.0342$). In addition, a statistical difference was observed between the brown control group and the green under thermal variation ($p = 0.0028$) and brown under thermal variation groups ($p = 0.0021$). The mean values were 8.09 µg/g in the green control group, 16.05 µg/g in the brown control, 3.67 µg/g in the green under thermal variation, and 3.04 µg/g in the brown under thermal variation, as shown in Fig. 5.

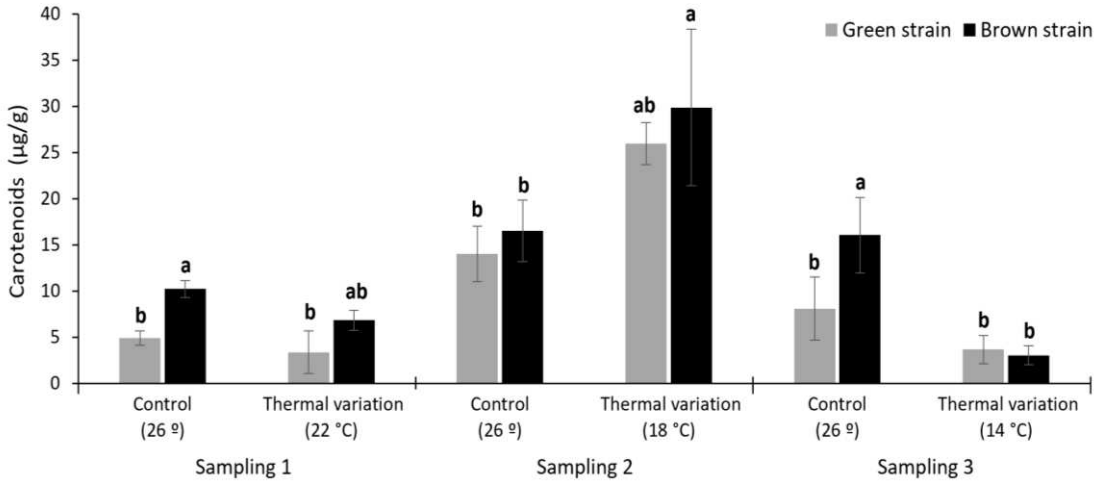


Fig. 5. Carotenoid levels (µg/g) of green and brown strains of *Kappaphycus alvarezii* cultivated at 26 °C (control) and under thermal variation, with samplings performed at 22 °C, 18 °C, and 14 °C. Bars represent mean ± standard deviation of triplicates. Statistical analyses were performed separately for each sampling, and different letters (a, b, ab) indicate significant differences among groups ($p < 0.05$, Tukey's test).

3.4 Phycobiliproteins

3.4.1 Phycoerythrin

At sampling 1, when the thermal variation samples were at 22 °C, there was a significant difference between the green under thermal variation group and the brown under thermal variation group ($p = 0.0134$). The mean values were 186.52 µg/g in the green control group, 318.87 µg/g in the brown control, 156.26 µg/g in the green under thermal variation, and 419.46 µg/g in the brown under thermal variation, as shown in Fig. 6.

At sampling 2, when the thermal variation samples were at 18 °C, there was a significant interaction between strain and treatment factors ($p < 0.0001$). A statistical difference was observed between the green control group and the brown control ($p < 0.0001$) and brown under thermal variation groups ($p = 0.008$). In addition, the brown control group showed a significant difference compared with the green under thermal variation ($p < 0.0001$) and brown under thermal variation groups ($p < 0.0001$), and a statistical difference was observed between the green under thermal variation and brown under thermal variation groups ($p = 0.0008$). The mean values were 182.87 µg/g in the green control group, 492.35 µg/g in the brown control, 147.47 µg/g in the green under

thermal variation, and 262.81 µg/g in the brown under thermal variation, as shown in Fig. 6.

At sampling 3, when the thermal variation samples were at 14 °C, there was a significant interaction between strain and treatment factors ($p = 0.0003$). A statistical difference was observed between the green control and brown control groups ($p < 0.0001$). The brown control group also showed a statistical difference compared with the green under thermal variation ($p < 0.0001$) and brown under thermal variation groups ($p < 0.0001$). In addition, a statistical difference was observed between the green under thermal variation and brown under thermal variation groups ($p = 0.0427$). The mean values were 111.41 µg/g in the green control group, 385.68 µg/g in the brown control, 43.86 µg/g in the green under thermal variation, and 119.93 µg/g in the brown under thermal variation, as shown in Fig. 6.

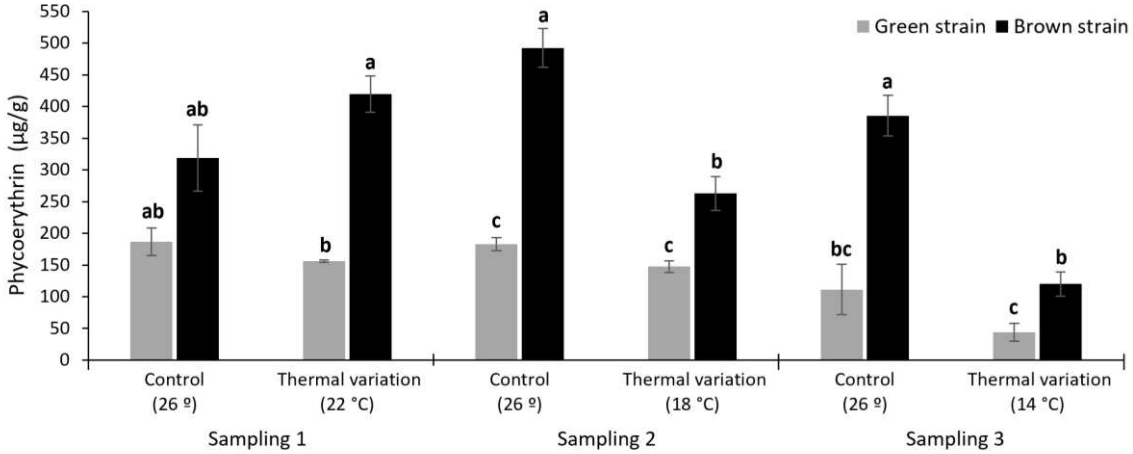


Fig. 6. Phycoerythrin levels (µg/g) of green and brown strains of *Kappaphycus alvarezii* cultivated at 26 °C (control) and under thermal variation, with samplings performed at 22 °C, 18 °C, and 14 °C. Bars represent mean ± standard deviation of triplicates. Statistical analyses were performed separately for each sampling, and different letters (a, b, c, ab, bc) indicate significant differences among groups ($p < 0.05$, Tukey's test).

3.4.2 Phycocyanin

At sampling 1, when the thermal variation samples were at 22 °C, there was a significant interaction between treatment and strain factors ($p = 0.0053$). A statistical difference was observed between the green control group and the brown control ($p = 0.0004$), green under thermal variation ($p = 0.0003$), and brown under thermal variation groups ($p < 0.0001$). The mean values were 171.56 µg/g in the green control group, 101.81 µg/g in the brown control, 97.54 µg/g in the green under thermal variation, and 79.32 µg/g in the brown under thermal variation, as shown in Fig. 7.

At sampling 2, when the thermal variation samples were at 18 °C, there was a significant difference between the green control group and the green under thermal variation ($p < 0.0001$) and brown under thermal variation groups ($p < 0.0001$). In addition, a statistical difference was observed between the brown control group and the green under thermal variation ($p = 0.0019$) and brown under thermal variation groups ($p = 0.0004$). The mean values were 185.17 µg/g in the green control group, 157.58 µg/g in the brown control, 107.60 µg/g in the green under thermal variation, and 95.30 µg/g in the brown under thermal variation, as shown in Fig. 7.

At sampling 3, when the thermal variation samples were at 14 °C, there was a significant difference between the brown control and brown under thermal variation groups ($p = 0.0119$). The mean values were 112.88 µg/g in the green control group, 145.43 µg/g in the brown control, 88.92 µg/g in the green under thermal variation, and 59.39 µg/g in the brown under thermal variation, as shown in Fig. 7.

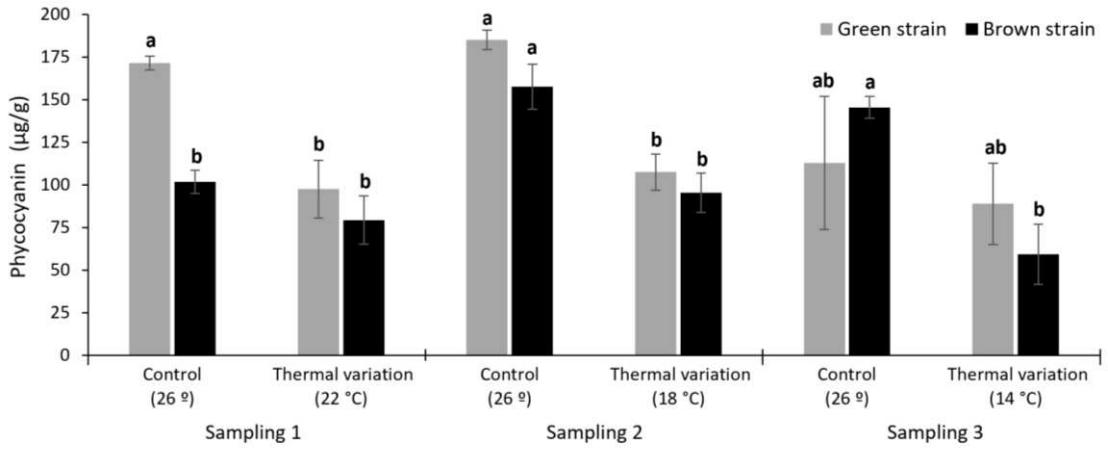


Fig. 7. Phycocyanin levels (µg/g) of green and brown strains of *Kappaphycus alvarezii* cultivated at 26 °C (control) and under thermal variation, with samplings performed at 22 °C, 18 °C, and 14 °C. Bars represent mean ± standard deviation of triplicates. Statistical analyses were performed separately for each sampling, and different letters (a, b, ab) indicate significant differences among groups ($p < 0.05$, Tukey's test).

3.4.3 Allophycocyanin

At sampling 1, when the thermal variation samples were at 22 °C, there was a significant difference between the brown under thermal variation group and the green control group ($p = 0.0395$). The mean values were 101.63 µg/g in the green control group, 135.92 µg/g in the brown control, 111.67 µg/g in the green under thermal variation, and 227.19 µg/g in the brown under thermal variation, as shown in Fig. 8.

At sampling 2, when the thermal variation samples were at 18 °C, there was a significant difference between the green control group and the brown control ($p = 0.0047$) and green under thermal variation groups ($p = 0.0124$). In addition, a statistical difference was observed between the brown control group and the green under thermal variation ($p < 0.0001$) and brown under thermal variation groups ($p = 0.0034$), as well as between the green under thermal variation and brown under thermal variation groups ($p = 0.0174$). The mean values were 168.24 µg/g in the green control group, 252.59 µg/g in the brown control, 96.88 µg/g in the green under thermal variation, and 163.90 µg/g in the brown under thermal variation, as shown in Fig. 8.

At sampling 3, when the thermal variation samples were at 14 °C, there was a significant difference between the brown control group and the green under thermal variation ($p = 0.0272$) and brown under thermal variation groups ($p = 0.0304$). The mean values were 135.29 µg/g in the green control group, 255.49 µg/g in the brown control, 93.94 µg/g in the green under thermal variation, and 97.48 µg/g in the brown under thermal variation, as shown in Fig. 8.

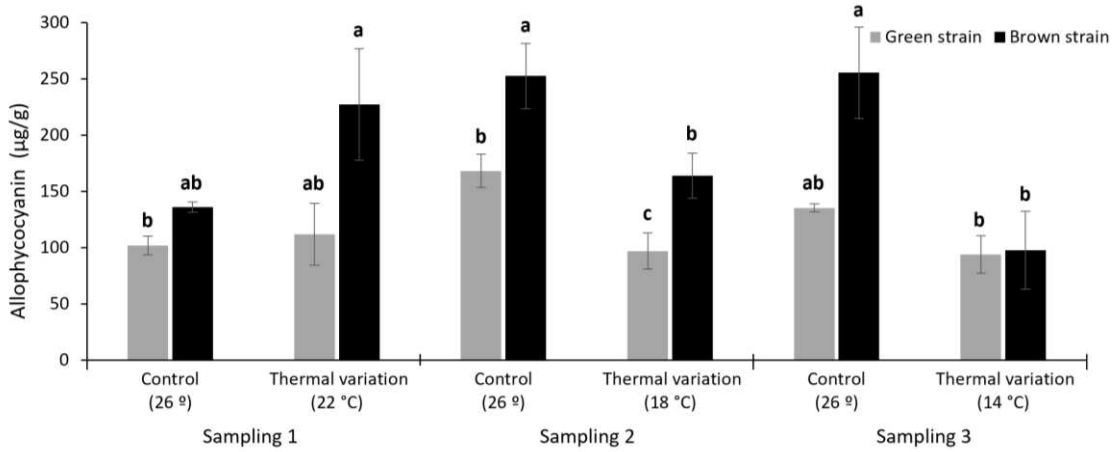


Fig. 8. Allophycocyanin levels (µg/g) of green and brown strains of *Kappaphycus alvarezii* cultivated at 26 °C (control) and under thermal variation, with samplings performed at 22 °C, 18 °C, and 14 °C. Bars represent mean ± standard deviation of triplicates. Statistical analyses were performed separately for each sampling, and different letters (a, b, c, ab) indicate significant differences among groups ($p < 0.05$, Tukey's test).

3.5 Total phenolic compounds

At sampling 1, when the thermal variation samples were at 22 °C, there was a significant difference between the green control group and the brown control ($p = 0.0005$) and brown under thermal variation groups ($p < 0.0001$). The brown control group also showed a statistical difference compared with the green under thermal variation group (p

= 0.0058). In addition, a statistical difference was observed between the green under thermal variation and brown under thermal variation groups (p = 0.0006). The mean values were 7.44 µg/g in the green control group, 16.74 µg/g in the brown control, 10.38 µg/g in the green under thermal variation, and 19.53 µg/g in the brown under thermal variation, as shown in Fig. 9.

At sampling 2, when the thermal variation samples were at 18 °C, there was a significant difference between the brown under thermal variation group and the green control group (p = 0.0129). The mean values were 6.67 µg/g in the green control group, 28.94 µg/g in the brown control, 14.41 µg/g in the green under thermal variation, and 39.84 µg/g in the brown under thermal variation, as shown in Fig. 9.

At sampling 3, when the thermal variation samples were at 14 °C, there was a significant difference between the brown under thermal variation group and the green control group (p = 0.0132). The mean values were 6.82 µg/g in the green control group, 15.96 µg/g in the brown control, 26.67 µg/g in the green under thermal variation, and 40.62 µg/g in the brown under thermal variation, as shown in Fig. 9.

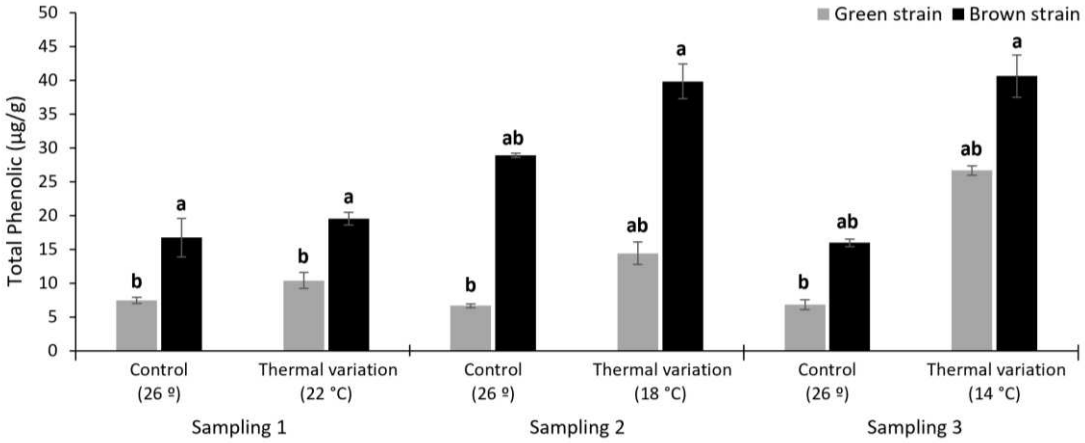


Fig. 9. Total phenolic compound levels (µg/g) of green and brown strains of *Kappaphycus alvarezii* cultivated at 26 °C (control) and under thermal variation, with samplings performed at 22 °C, 18 °C, and 14 °C. Bars represent mean ± standard deviation of triplicates. Statistical analyses were performed separately for each sampling, and different letters (a, b, ab) indicate significant differences among groups ($p < 0.05$, Tukey's test).

4 DISCUSSION

Seaweed cultivation is a sustainable activity that supports the economy of coastal communities in various regions worldwide, including the Brazilian coast. In Santa Catarina, the production of *K. alvarezii* has gained prominence, mainly due to the use of its biomass for the extraction of biostimulants. However, the low water temperatures recorded during winter can compromise productivity. According to EPAGRI (personal

communication), in 2022 and 2023 the water temperature in the region of Florianópolis ranged from 20 °C to 15 °C.

In this context, understanding the effects of thermal stress on the cultivation of *K. alvarezii* is essential, since temperature directly influences photosynthesis, metabolism, and respiration, acting as one of the main determinants of seaweed growth (Bulboa and Paula 2005; Gelli et al. 2024). Isniwati et al. (2025) emphasized that the range of 26–32 °C favors the development of the species and highlighted the importance of adequate thermal conditions to ensure productivity in subtropical regions.

Growth rates above 3% day^{-1} are considered satisfactory for the cultivation of *K. alvarezii* (Isniwati et al. 2025). In this study, thalli cultivated at 26 °C in the control group exceeded this reference value, indicating that this temperature favors the development of the species. In contrast, Hayashi et al. (2011) demonstrated that although the species can grow at 16 °C, rates remain low, with values of 0.32–0.54% day^{-1}, a pattern also confirmed in our experiments.

Under thermal variation, growth rates progressively decreased in both strains. At 18 °C, the brown strain showed a rate of 1.08% day^{-1}, significantly lower than both its control group, 4.27% day^{-1}, and the green control group, 3.49% day^{-1}. At 16 °C, the reduction was even more pronounced, with growth of 0.36% day^{-1} compared with 4.72% day^{-1} in the control group. At 14 °C, the brown strain exhibited only 0.07% day^{-1}, significantly lower than the green control group but statistically similar to its own control group, % day^{-1}, underscoring its strong sensitivity to thermal stress.

In contrast, the green strain at 24 °C showed a mean growth rate of 5.24% day^{-1}, significantly lower than its control group, 9.49% day^{-1}. At other temperatures, no significant differences were observed compared with the control groups, although rates dropped to 0.88% day^{-1} at 18 °C versus 4.72% day^{-1} and to only 0.34% day^{-1} at 14 °C versus 4.51% day^{-1}. This pattern indicates that both strains were affected by decreasing temperature; however, the green strain performed better than the brown, whose growth rate was nearly zero. Moreover, the green strain accumulated more biomass in the control group, which filled much of the container in the final weeks, reinforcing the productive potential of *K. alvarezii* under favorable temperatures, though potentially limiting further growth (Cáceres-Farias et al. 2025).

The regression curves, Supplementary information (Fig. S1 A, B) support these results. Under thermal variation, the brown strain showed a sharp decline from 22 °C, reaching values near zero at 14 °C, whereas the green strain exhibited a more gradual

reduction. In the control group, both strains maintained more stable rates over time, with the green outperforming the brown during most of the cultivation period. These findings corroborate the field study of Costa et al. (2024), in which the daily growth rate of *K. alvarezii* was positively correlated with water temperature, and the green strain performed better during winter, confirming its greater resilience to cold.

In this study, a gradual decrease in temperature led to a sharp reduction in growth rates in both strains, with the lowest values recorded at 14 °C. The green strain maintained greater growth stability, whereas the brown strain showed more pronounced reductions. These results are consistent with (Araújo et al. 2014), who reported greater plasticity of the green strain under temperature and salinity variations, and with Cáceres-Farias et al. (2025), who highlighted the higher sensitivity of the red and brown strains under unstable environmental conditions.

A temperature of 14 °C can be considered a critical threshold for the development of *K. alvarezii*, as corroborated by histological findings showing cell wall thickening in samples cultivated at 14 and 18 °C, mainly in the brown strain. Similarly, Schmidt et al. (2010) reported that, after exposure to UV-B radiation, *K. alvarezii* strains exhibited increased cortical cell wall thickness, interpreted as a defense mechanism against ultraviolet radiation. This finding is consistent with what was observed in the present study, where cell wall thickening can be interpreted as an adaptive response to the stress imposed by low temperatures.

Beyond these effects, several studies have reported that thermal stress directly affects the metabolism and physiology of *K. alvarezii*. In the present study, analyses of photosynthetic pigments at three sampling points (22 °C, 18 °C, and 14 °C), compared with the control at 26 °C, revealed a reduction in the final weeks under thermal variation, especially at 14 °C, as illustrated in the figures. Similarly, Narvarte et al. (2022) reported a decrease in pigments after 42 days of cultivation at 20 °C, indicating that, although experimental conditions differed, pigment loss consistently reflects a physiological response to stress.

Chlorophyll *a* is the main photosynthetic pigment in seaweed, essential for photosynthesis but sensitive to environmental changes (Schmidt et al. 2010). In this study, a significant difference was detected only at 14 °C, when both strains under thermal variation showed the lowest chlorophyll *a* levels, in contrast to the brown control group, which maintained the highest values. This finding indicates greater preservation of photosynthetic activity under optimal thermal conditions, such as 26 °C. Similarly,

Syahrul et al. (2023) reported higher chlorophyll *a* levels in *K. alvarezii* cultivated at 28 °C and 31 °C, with a marked reduction at 34 °C. Other studies have also confirmed that environmental stress causes pigment degradation, reduced photosynthesis, and decreased chlorophyll *a* in *K. alvarezii* (Kumar et al. 2020). Moreover, the literature suggests that chlorophyll accumulation in this species is associated with biomass increase and chloroplast development (Paransa et al. 2020). Thus, the absence of growth in the groups under thermal variation reinforces the direct relationship between environmental stress and impaired photosynthetic activity.

Carotenoids, another pigment analyzed, function as accessory pigments and play a role in the photoprotection of *K. alvarezii*. Significant differences were observed at 22 °C, with the brown control group exhibiting the highest levels compared with the green strain, both in control and under thermal variation. In contrast, at 18 °C, the brown strain showed higher levels than in the controls. At 14 °C, only the brown control maintained higher values, differing from all others and indicating carotenoid degradation under pronounced cold stress. These findings suggest that carotenoid synthesis differs between strains and that the brown strain demonstrates a more effective protective response under stress, reinforcing the ability of seaweed to modulate bioactive compounds according to environmental conditions and strain-specific tolerance to thermal fluctuations (Mekinić et al. 2023; Gelli et al. 2024). Moreover, according to (Li et al. 2016b), the photosynthetic processes of *K. alvarezii* are highly sensitive to cold, which promotes enzymatic degradation and damage to the photosynthetic apparatus through the accumulation of reactive oxygen species (ROS). This oxidative imbalance helps explain the decrease in chlorophyll and carotenoids at low temperatures observed in the present study.

In addition to chlorophyll and carotenoids, red algae contain phycobiliproteins (phycoerythrin, phycocyanin, and allophycocyanin), which function as accessory pigments in photosynthesis. These pigments are organized into structures called phycobilisomes, located on the outer surface of the thylakoids, that absorb light mainly in the 500-650 nm range (Liu et al. 2005). Phycobilisomes act as antennas associated with photosystem II, capturing light energy and transferring it to chlorophyll, where photosynthesis begins (Grossman et al. 1993; Singh et al. 2015). In addition to their photoprotective role, phycobiliproteins may also serve as nitrogen reserves, with reduced levels of phycoerythrin being associated with the lower nitrogen storage capacity of the green strain compared with the red one (Dawes 1992), while increases in phycocyanin and allophycocyanin indicate greater nitrogen accumulation.

Our findings showed that pigment responses differed between strains and treatments, with the brown strain generally exhibiting higher levels of phycoerythrin, phycocyanin, and allophycocyanin under control conditions, whereas low temperatures compromised their stability. This effect is consistent with previous reports that thermal stress can disrupt energy capture and transfer processes, leading to reduced concentration and stability of pigments (Nouri et al. 2015). Significant interactions were detected for phycoerythrin (18 °C and 14 °C) and for phycocyanin (22 °C), indicating that the physiological response results from the combination of these factors and reinforcing that cold tolerance is strain dependent.

The ability of the brown strain to preserve pigments may be linked to the action of other antioxidant components, such as phenolic compounds. In this study, the brown strain showed higher phenolic levels at 22 °C compared with the green strain under the same condition and in the control group. A similar pattern was observed at 18 °C and 14 °C, when the highest levels were again recorded in the brown group relative to the green control group, suggesting stronger activation of this antioxidant pathway in response to thermal stress. These differences in phenolic content between strains may reflect distinct responses to oxidative stress. In particular, the greater accumulation detected in the brown strain at low temperatures can be mechanistically explained by a slowdown of the Calvin cycle due to reduced Rubisco activity (Li et al. 2016b). This process leads to the accumulation of unused NADPH and ATP, increasing ROS formation and raising the risk of damage to membranes, proteins, and pigments, as well as impairing photosystem II repair (Li et al. 2016b). In this context, phenolics likely act synergistically with enzymatic antioxidant mechanisms, contributing to the maintenance of cellular homeostasis.

According to the above, the brown strain stood out for preserving pigments under stable temperatures and for accumulating higher phenolic levels in response to cold, whereas the green strain maintained greater physiological stability at lower temperatures. Together, these findings underscore that selecting cold tolerant genotypes, as well as cultivating strains in combination, represents a promising strategy to optimize productivity and minimize seasonal losses in regions with harsh winters.

5 CONCLUSION

The gradual reduction in temperature impaired the growth of *K. alvarezii*, with a marked decline in growth rates from 18 °C and minimum values at 14 °C, accompanied

by cell wall thickening, more evident in the brown strain and occurring in the green strain only under extreme cold. The green strain maintained greater growth stability, whereas the brown strain preserved photosynthetic pigments more effectively under control conditions and accumulated higher levels of phenolic compounds under cold stress. Significant strain × treatment interactions were detected for phycobiliproteins, indicating that the response to thermal stress results from the combination of these factors. In summary, the physiological, morphological, and biochemical differences reinforce the importance of selecting cold tolerant strains to optimize productivity and reduce seasonal risks in regions with harsh winters.

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AUTHORS' CONTRIBUTIONS

Jaqueline da Rosa Coelho conducted the experiments and performed all analyses, including pigments, phenolics, optical microscopy, statistics, and manuscript writing. Luciana Alves Espírito Santo and Fernando Zwierzikowski da Silva assisted in experimental management. Carmen Simioni contributed to experimental design, supported data analysis, and revised the manuscript, including the final version. Thallis Felipe Boa Ventura assisted with phenolic analyses. Felipe Boéchat Vieira provided resources. Scheila Anelise Pereira Dutra contributed to experimental design, resources, supervision, and final revision of the manuscript. All authors approved the final version and are accountable for the integrity of the work.

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DATA AVAILABILITY STATEMENT: Data will be made available on request.

CONFLICT OF INTEREST: The authors declare that they have no conflict of interest.

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