

Biomass production of the red macroalga *Gracilariopsis tenuifrons* (Gracilariales) using a low-cost commercial fertilizer and its additional benefits for carbon sequestration

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Research Article

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Abstract

Gracilariaceae species are economically good options for aquaculture in Brazil. High-quality seedlings produced by vegetative reproduction is necessary to decrease pressure on natural beds and can be achieved in controlled environments. Commercial fertilizers are a low-cost option to increase quality and growth. Furthermore, CO₂ sequestration can be an added value to cultivation. This study aimed to analyze the biomass production and CO₂ sequestration of *Gracilariopsis tenuifrons* (C.J.Bird & E.C.Oliveira) Fredericq & Hommersand under two concentrations of a low-cost commercial fertilizer. Growth, pigment content, proportion of dry biomass, C, H, and N contents, and CO₂ sequestration were estimated. *Gracilariopsis tenuifrons* were kept for 28 days under two concentrations of the commercial orchid fertilizer Forth 30-10-10 (NPK), one with 250 µM of N (called 50%) and the other with 500 µM of N (called 100%). No significant differences were found in growth and growth rate between 50% and 100%, with maximal 4.4-times biomass at the end of the experiment. Significant increases were found in the concentrations of phycobiliproteins and N, which were 33% and 20% higher in the samples with Forth 100%, respectively. The C content was higher in the Forth 50% samples, with a CO₂ fixation of 14.8% per unit of fresh mass. *Gracilariopsis tenuifrons* showed a CO₂ sequestration rate equivalent to that of other macroalgae species, demonstrating its potential in culture. In addition, greater biomass production and CO₂ capture could be stimulated by combining the addition of nutrients with higher luminosity to increase the rate of photosynthesis and growth.

1. Introduction

Marine macroalgae, or seaweeds, encompass a great diversity of organisms including representatives of three lineages, red algae (Rhodophyta), green algae (Chlorophyta), and brown algae (Ochrophyta, Phaeophyceae). They form the most extensive and productive coastal habitat in the world, covering an area of around 3.4 million km² (Krause-Jensen et al. 2018). Macroalgae beds perform various services and provide several important goods for the ecosystem in terms of supply, regulation, support, and cultural functions, serving as subsistence for millions of people and habitats for various marine organisms, absorbing and cycling nutrients, and protecting against ocean acidification and deoxygenation (Sondak et al. 2017). As photosynthesizing organisms, they also contribute to the carbon cycle by fixing CO₂. Their high net primary production supports around 1.32 x 10⁹ tons of carbon per year (Duarte et al. 2022), of which 11% is potentially sequestered in the sediment or deep sea, where carbon is kept for hundreds to thousands of years (Krause-Jensen et al. 2018).

For the past 50 years, macroalgae aquaculture has become one of the fastest-growing sectors in the world, with an average growth rate of 8% per year (Duarte et al. 2017), and now accounts for nearly 30% of all aquaculture production worldwide. In 2019, there was a total production of 35.7 million tons of fresh biomass, 99.1% of which was produced on the Asian continent. However, most seaweed cultivation relies on a small number of genera, such as the brown algae *Saccharina* and *Undaria*, and the red algae *Kappaphycus*, *Eucheuma*, *Gracilaria*, and *Pyropia* (FAO 2021). The produced macroalgal biomass is used

by diverse sectors of society, such as the phycocolloid industry, human food, fertilizers for agriculture, industrial, pharmaceutical, or cosmetic applications, and biofuels (Sondak et al. 2017). Although current production is nowhere near the size of natural beds, there is great potential for expansion in this sector. A survey by Spillias et al. (2023) found an area of approximately 6.5 million km² ecologically available for macroalgae aquaculture, compared to the 1,900 km² cultivated in 2014.

Gracilarioids species, red seaweeds from the Gracilariaceae family, are widely used commercially for food and especially for extracting agar. According to FAO (2021), they accounted for around 10% of the world's macroalgae production in 2019 and had the third highest produce value per kilo (USD 0.54/kg). The greatest specific diversity occurs in the genera *Gracilaria* (199 species) and *Gracilariopsis* (24 species), which together produce approximately 91% of all agar (Porse and Rudolph 2017). Agar is a high-value product used in many industries such as food, cosmetics, pharmaceuticals, and textile, due to its emulsifying, stabilizing, and gelling properties (Zhang et al. 2024). In 2015, it had a market size of USD 246 million with an average growth rate of 7% (Porse and Rudolph 2017). Moreover, in recent studies, the sulfated constituent of agar has been evidenced with diverse biological activity, including antioxidant, antiviral, antitumor, and anti-inflammatory properties (Torres et al. 2019).

The Brazilian coast is home to more than 800 species of seaweeds, 62% of which are Rhodophyta. Species from *Gracilaria*, *Gracilariopsis*, and *Hypnea* have been exploited in the northeast region since 1960 and for many years were an important source of income for local communities, which would export the biomass to Japan (Marinho-Soriano 2017). However, overexploitation of the natural beds led to a drop in seaweed availability and consequently a decline in local supply and exports to negligible amounts. In the following years, *Gracilaria birdiae* E.M. Plastino & E.C. Oliveira started to be locally cultivated in the states of Paraíba, Rio Grande do Norte, and Ceará, but its reliance on natural beds for seedlings risked uncontrolled harvesting (Alemañ et al. 2019). Nowadays, the seaweed aquaculture industry in Brazil is still in its infancy, with the exotic carrageenophyte species *Kappaphycus alvarezii* (Doty) L.M. Liao being the first with a commercial farming scale in the south and southeast Brazilian regions. However, with its 8,000 km coastline and favorable environmental conditions, Brazil has great potential for seaweed aquaculture (Barbosa-Silva et al. 2023).

As observed in the northeast of Brazil, industrial dependency on natural beds for biomass harvest is unsustainable for both the maintenance of wild stocks and for supplying the growing demand for seaweed material. Also, it is often seen as a cause of market volatility (Gupta et al. 2018). Therefore, seaweed cultivation in the sea can thus reduce the pressure of extractivism on natural populations and ensure the production of macroalgae for various sectors of society. However, another challenge related to the seaweed biomass supply chain is the production of seed materials for farming, since many producers depend on seed stocks harvested from natural beds (Jiksing et al. 2022). On top of that, as the seaweed industry continues to grow, also increases the demand for biomass which the existing resources cannot meet. Hence, creating an uninterrupted supply of seedlings for cultivation is essential to manage the growing market demands and the seaweed industry in Brazil (Naik and Naik 2020).

Many strategies for seedling production have been proposed by the literature, the two most common ones through vegetative fragments and reproductive cells (Jiksing et al. 2022). The former is recommended for species with higher proliferation potential, such as *Kappaphycus*, *Gracilaria*, and *Gelidium*, while the latter is for species with greater reproductive potential, such as *Pyropia*, *Saccharina*, and *Undaria* (Gupta et al. 2018). Clonal propagation through vegetative fragments has the advantage of having rapid regeneration, better survival, and uniform productivity, as well as having a shorter culture time and lower costs compared to reproductive techniques (Jaiswar et al. 2021). Moreover, it helps avoid overexploitation of natural beds through thallus reuse. On the other hand, it is believed that it can lead to decreased genetic variability, lower growth rates, and higher vulnerability to pathogens, due to clonal repetition (Arbaiza et al. 2023).

The problems related to vegetative reproduction could be alleviated by laboratory cultivation methods in controlled environments and by selecting fast-growing, disease-resistant plantlets from different parental lines (Radulovich et al. 2015). The possibility of cultivating seedlings in stable environmental conditions could enhance productivity and allow species-specific protocols, in contrast to cultivation in the sea, where conditions are unstable and unpredictable. Furthermore, clonal propagation could inhibit reproductive development, leading to higher growth rates due to less energy being used for reproduction (Saminathan et al. 2015). Consequently, a higher yield and quality could be obtained, allowing a continuous and reliable supply of quality seed material.

In a cultivation system, nutrient availability is one of the key factors controlling the primary production of macroalgae, with phosphorus and nitrogen being the main limiting nutrients, as they are crucial elements for energy metabolism and the production of structural molecules (Barbosa-Silva et al. 2023). Culture media used for laboratory experiments are normally expensive and complex to produce and, therefore, cannot be used for large-scale seaweed cultivation. As an alternative, commercial fertilizers are a cheaper and more viable option, which are also able to provide the nutrients required by seaweed (Fernandes et al. 2017). However, since different species show different responses to nutrient concentration (Murakami and Rodrigues 2009), it is essential to study the nutritional needs of different groups. Hence, as a first step towards understanding nutritional demands and their influence, laboratory experiments under controlled conditions are important to estimate physiological patterns, phenotypic plasticity, and tolerance and stress limits, which will allow cultivation success.

Several studies have described the macroalgae aquaculture potential for carbon sequestration and as an additional help to mitigate greenhouse gas (GHG) emissions (Chung et al. 2011; Sondak et al. 2016; Duarte et al. 2017; Krause-Jensen et al. 2018). Moreover, including this aspect in the cultivation process may represent an additional income for producers, since it could be incorporated in carbon credit markets. However, its use as a potential carbon sequester depends on the destination of the produced biomass. For instance, applications such as food, animal feed, or biofuels do not work as long-term storage, since the CO₂ is re-emitted into the atmosphere (Sondak et al. 2017), while its use as biochar or to produce biopolymers could represent longer-term storage. On the other hand, even temporary carbon storage through seaweed products could be beneficial to the climatic system by substituting other

products with associated carbon emissions or by possible carbon flows from seaweed cultivation sites to the deep ocean (Hasselström and Thomas 2022).

In the case of seedling cultivation on land, using carbon sequestration as an added value to the process could help alleviate some of the problems related to this method, such as the high costs of infrastructure and maintenance, as well as high energy and water consumption (Jiksing et al. 2022), increasing, therefore, its economic viability. However, since carbon content, dry weight rate, and biomass yield vary between species and environmental conditions, it is essential to study different species with economic value in controlled environments, to create cultivation protocols that maximize growth and quality.

Accordingly, the cultivation of Gracilariaceae species could be an option to increase Brazil's seaweed industry, more specifically for agar, or biopolymer and bioactive compounds applications, and could be additionally used as an important tool for reducing GHG emissions and mitigating the effects of global climate change. Furthermore, the development of cultivation farms will depend on a constant supply of high-quality seedlings, provided through vegetative fragments cultivated in controlled environments such as laboratories. Also, the application of commercial fertilizers could be used as an extra tool to increase growth and quality, as well as decrease production costs. Finally, accounting for CO₂ sequestration during production can act as an added value that increases the economic viability of cultivation.

The red alga *Gracilariopsis tenuifrons* (C.J. Bird & E.C. Oliveira) Fredericq & Hommersand is found in Brazil from the states of Ceará to São Paulo (Oliveira 1998; Oliveira et al. 2002) and is also present in Mexico, Cuba, and Venezuela (Faria 2022). It is considered to have a high growth rate and produces good quality agar (Brito and Silva 2004). In addition, it can be used as a biofilter to absorb nutrients from aquaculture, such as marine fish and shrimp (Hernández et al. 2006; Carneiro et al. 2021), is easy to grow in the laboratory and has the potential for large-scale production in Brazil (Plastino 1991; Oliveira 1998). This research aims to analyze the biomass production and CO₂ sequestration potential of *G. tenuifrons* under laboratory cultivation of two concentrations of a low-cost commercial fertilizer. We aspire to contribute to future RD&I projects that value *G. tenuifrons* in multiphase exploitation dimensions and add value to this species' cultivation activity.

2. Material and methods

2.1. Biological material and general culture conditions

The female gametophyte of *G. tenuifrons* was collected from the Southeast Brazilian coast at Cibratel Beach in Itanhaém city, São Paulo State (24°13'31" S, 46°51'7" W), and was established in laboratory cultivation to produce stock material and subsequent utilization in the experiment. The stock was maintained in von Stosch-enriched seawater (VS, Table 1; Ursi and Plastino 2001) diluted to 50% with sterile seawater (salinity at 32), corresponding to 250 µM of nitrogen. The biomass-to-medium ratio was 3 g of fresh weight (FW) per 1 L of medium, while maintaining controlled conditions of temperature at 25

$\pm 1^{\circ}\text{C}$ with 14 h photocycle, irradiance of $60 \pm 5 \mu\text{mol photons m}^{-2} \text{s}^{-1}$, and intermittent aeration for 30 min.

Table 1
Composition of the von Stosch enrichment solution (VS)
(Ursi and Plastino (2001), modified from Edwards (1970))

Additive	von Stosch
NaNO ₃	0.50 mM
Na ₂ HPO ₄ .12H ₂ O	30.00 μM
MnSO ₄ .H ₂ O	0.10 μM
FeCl ₃ .6H ₂ O	1.15 μM
Thiamine	0.59 μM
Biotin	4.10 nM
Cyanocobalamin	0.74 nM
Ethylenediaminetetraacetic acid (EDTA)	12.70 μM

The seawater used was double-filtered using CUNO filters with pore sizes of 2 μm and 1 μm , followed by sterilization through ionizing radiation (UV-C lamp, QUIMIS Q884-21 system; 3.8 L min^{-1}). Irradiance was provided by tubular LED lamps (6500 K/18 W) and measured with a LI-COR quantum sensor (LI-250, USA) and a LI-COR spherical sensor (LI-193, USA). Aeration was supplied by an oil-free diaphragm compressor (IBRAM CR03).

2.2. Experimental design

From the stock material, apical segments of 4 cm in length were cultivated for 28 days under two enrichment conditions: (a) Forth solution corresponding to a concentration of 100% of the VS solution (500 μM) and (b) Forth solution corresponding to a concentration of 50% of the VS solution (250 μM), with five biological replicates per treatment, at a biomass-to-medium ratio of 1 g FW per 1 L of medium. The commercial orchid fertilizer Forth 30-10-10 (N-P-K) was selected due to the elevated nitrogen concentration, one of the most important nutrients for red macroalgae.

Every week, the culture medium was changed and the material was weighted, to record the biomass and calculate the growth parameters. After 28 days, subsamples of each replicate were collected, frozen in liquid nitrogen (N₂), and stored at -80°C to be used for chemical and other analysis.

2.3. Analytical procedures

Growth, growth rate, and duplication coefficient were assessed weekly (at times T1, T2, T3, and T4, corresponding to each week). The same parameters were assessed considering the accumulated

growth level. The growth rate was calculated using the formula described by Lignell and Pedersén (1989) and the duplication coefficient as the ratio between final biomass and initial biomass.

The algal pigment contents of phycobiliproteins, chlorophyll-a (Chl-a), and total carotenoids were analyzed using 50 mg FW. The procedure followed the descriptions of Beer and Eshel (1985) with minor modifications. Samples were ground in liquid N₂ until a fine powder was obtained. Subsequently, the material was suspended for 1 h in 1.5 mL of 0.1 M sodium phosphate buffer (pH 6.8) and kept in the dark at 4°C. After the suspension, the extracts were centrifuged at 12,000 rpm and 4°C for 15 min. The supernatant was collected for phycobiliproteins analysis. The remaining sedimentary material was suspended for 1 h in 1.5 mL of methanol and centrifuged again at 12,000 rpm and 4°C for 15 min. Its supernatant was collected for Chl-a and total carotenoids analysis.

Absorbance readings were performed on a spectrophotometer scanning BioTek Epoch UV-vis microplate reader (USA) with a 1 cm optical path length. Phycobiliproteins were obtained following the formulae of Beer and Eshel (1985) and standardized to the FW and the extraction volume as: PE ($\mu\text{g g FW}^{-1}$) = $[(\text{Abs}564 - \text{Abs}592) - (\text{Abs}455 - \text{Abs}592) \times 0.2] \times 0.12 \times [\text{volume (mL)} / \text{FW (g)}]$ and PC ($\mu\text{g g FW}^{-1}$) = $[(\text{Abs}618 - \text{Abs}645) - (\text{Abs}592 - \text{Abs}645) \times 0.51] \times 0.15 \times [\text{volume (mL)} / \text{FW (g)}]$. Where PE = phycoerythrin, PC = phycocyanin, and Abs = absorbance at the corresponding wavelength. Chlorophyll-a and total carotenoids were determined following the formulae of Jeffrey and Humphrey (1975) and Wellburn (1994) respectively and standardized to the FW and the extraction volume as: Chl-a ($\mu\text{g g FW}^{-1}$) = $11.0607 \times \text{Abs}664 \times [\text{volume (mL)} / \text{FW (g)}]$ and total carotenoids ($\mu\text{g g FW}^{-1}$) = $(1000 \times \text{Abs}480 - 0.89 \times \text{Chl-a}) / 245 \times [\text{volume (mL)} / \text{FW (g)}]$.

The dry biomass data were obtained by weighing the samples (approximately 1.3 g FW) placed in an oven for 4 days at a temperature of 60°C. The ratio of dry mass to fresh mass was calculated by dividing the dry mass by the fresh mass of each sample and then expressed as a percentage. Following the weighing, the pulverized oven-dried material was analyzed for elemental quantification of CHN using the Perkin Elmer (USA) model 2400 CHN elemental analyzer by the Analytical Center facility of the Institute of Chemistry at the University of São Paulo. The dried samples were carbonized at 926°C in the presence of pure oxygen to oxidize the material. As a result, carbon dioxide (CO₂) was obtained from the carbon in the samples, water from the hydrogen, and oxides (N_xO_x) from the nitrogen, which was then reduced to nitrogen gas (N₂). The products were carried by helium gas to a reduction chromatographic column (at 640°C), where the elements were separated and detected by thermal conductivity. The results were calculated based on the quantities of elements obtained in the CHN analysis and expressed as percentage.

The CO₂ fixation data were estimated from the results of the tissular carbon content in the dry biomass. For this purpose, the carbon per dry mass data was multiplied by a factor of 3.67, representing the molecular weight ratio of CO₂ to carbon (Pendleton et al. 2012; Sondak et al. 2017). The values were expressed in grams of CO₂ per gram of dry mass. Furthermore, the CO₂ data for each sample were

multiplied by the values of the ratio of dry biomass to fresh biomass to obtain the values of CO₂ per FW, expressed as a percentage.

2.4. Statistics

Growth, growth rate, and duplication coefficient were analyzed by one-way ANOVA, followed by the *a posteriori* Tukey test. Pigment concentrations (PE, PC, Chl-a, and total carotenoids), dry biomass ratio, CHN content, and CO₂ fixation were analyzed using the Student's t-test. Statistical analysis was performed using the PAST 4.03 software, considering a 95% confidence interval ($p < 0.05$).

3. Results

The overall appearance including size, branching, color, and visual biomass of *G. tenuifrons* under both nutritional Forth treatments showed no differences over the experimental period (Fig. 1). Only the accumulation of biomass over time was observable in both treatments.

The growth and duplication coefficient followed an increasing trend over time (Fig. 2a), without difference between the Forth treatments. The coefficient at the end of the experiment was 4.38 ± 0.26 times for Forth 50% and 4.34 ± 0.42 times for Forth 100%. Every 2 weeks, a double duplication coefficient was observed. Then, the biomass more than quadrupled during the experimental period and under the studied conditions. From the initial T0 time with 0.300 g of biomass, it achieved a maximum growth of about 1 g after 4 weeks. Similarly, non-difference between the Forth treatments was registered for the growth rate over time and for the total growth rate (Fig. 2b), ranging from $5.37\% \text{ day}^{-1}$ (Forth 100%) to $5.41\% \text{ day}^{-1}$ (Forth 50%). Regarding the experimental period, the highest growth rate was evidenced at T2 (around $7.46\% \text{ day}^{-1}$), followed by a stable rate of around $5\% \text{ day}^{-1}$.

Phycoerythrin (*t*-test: $p = 0.037$) and phycocyanin (*t*-test: $p = 0.035$) analyses revealed significant differences between Forth 50% and Forth 100% treatments (Fig. 3a) with higher phycobiliproteins in samples subjected to Forth 100%. The mean concentration of phycoerythrin with Forth 50% was $953.96 \pm 348.99 \mu\text{g g FW}^{-1}$, approximately 33% lower than that observed with Forth 100% ($1436.44 \pm 255.28 \mu\text{g g FW}^{-1}$). Similarly, phycocyanin values followed the same trend, with Forth 50% ($102.77 \pm 35.13 \mu\text{g g FW}^{-1}$) being approximately 33% lower than Forth 100% ($153.69 \pm 28.30 \mu\text{g g FW}^{-1}$).

On the other hand, results for chlorophyll-a (*t*-test: $p = 0.555$) and total carotenoids (*t*-test: $p = 0.545$) did not show significant differences between treatments (Fig. 3a). The chlorophyll-a content was $230.57 \pm 40.29 \mu\text{g g FW}^{-1}$ for Forth 50% and $250.24 \pm 59.07 \mu\text{g g FW}^{-1}$ for Forth 100%. The quantities of carotenoids were $15.20 \pm 2.05 \mu\text{g g FW}^{-1}$ and $17.10 \pm 6.41 \mu\text{g g FW}^{-1}$, respectively. As a typical rhodophyte, the phycoerythrin content was extremely elevated compared to other accessory pigments and even to chlorophyll-a.

Dry biomass of *G. tenuifrons* under both nutritional Forth treatments showed no significant differences between treatments (*t*-test: $p = 0.753$). Samples receiving Forth 50% had an average of $15.11 \pm 0.30\%$ dry

mass, while those with Forth 100% exhibited $15.20 \pm 0.52\%$ dry mass.

CHN analyses revealed significant differences for carbon (t -test: $p = 0.033$) and nitrogen (t -test: $p = 0.000$), and no differences were registered for hydrogen (t -test: $p = 0.180$) (Fig. 3b). Carbon values for Forth 50% sample was $26.93 \pm 0.70\%$, while Forth 100% sample had $26.30 \pm 0.51\%$ carbon. Nitrogen data for algae with Forth 50% ($3.07 \pm 0.32\%$) were approximately 20% lower than the values for algae with Forth 100% ($3.84 \pm 0.43\%$). Finally, hydrogen values for Forth 50% sample wase $4.67 \pm 0.28\%$, whereas those with Forth 100% presented $4.48 \pm 0.32\%$.

Estimated CO₂ fixation of *G. tenuifrons* revealed significant differences between treatments only for the dry weight data (t -test: $p = 0.033$). In contrast, results per fresh weight showed no significant differences (t -test: $p = 0.296$) (Fig. 3c). The proportion of fixed CO₂ for samples with Forth 50% was $14.94 \pm 0.59\%$ FW⁻¹ (0.99 ± 0.03 g g DW⁻¹), while those with Forth 100% obtained a proportion of $14.66 \pm 0.48\%$ FW⁻¹ (0.97 ± 0.02 g g DW⁻¹) of CO₂. Table 2 presents the average CO₂ fixation for *G. tenuifrons* in this experiment (14.8% FW⁻¹) and compares it to the CO₂ fixation of other species from the literature (Sondak et al. 2016).

Table 2
CO₂ sequestration (% FW⁻¹) of Gracilariopsis tenuifrons and other marine macroalgae

Species	CO ₂ sequestration (% FW ⁻¹)*
<i>Kappaphycus</i> spp.	11.4–23.7
<i>Gelidium</i>	19.8–22.0
<i>Sargassum</i>	18.2–20.4
<i>Ecklonia</i>	17.6–18.7
<i>Saccharina</i>	13.8–17.1
<i>Pyropia</i>	15.0
Gracilariopsis tenuifrons	14.8
<i>Gracilaria</i>	13.0
<i>Undaria</i>	13.0
* From the carbon content data, the conversion was made using 15% as the standard value for the proportion of dry biomass and the factor 3.67 for the transformation into CO ₂ (Sondak et al. 2017).	

Discussion

Factor limiting such as non-nitrogen nutrients, light, pH, or temperature usually directly affect seaweeds' growth rate (Perdersen and Bórum 1996). The lack of difference in growth of our results under the tested

treatments indicates that a limiting condition other than nitrogen may have occurred. Furthermore, according to Ribeiro et al. (2013), red algae are known for storing nitrogen in the form of proteins, pigments, and amino acids when it is abundant in the environment, so it is not used in its entirety for the alga's growth. This scenario could change, for example, with increased luminosity, leading to greater photosynthesizing activity and, therefore, a greater nutritional requirement.

On the other hand, our results also show that the use of Forth 100% did not represent an increase in production. Therefore, using Forth 50% would be enough to achieve the same results with half the amount of fertilizer, which could enhance the economic viability of seedling production on land. This could be related to the fact that some marine algae adapted to oligotrophic tropical waters show positive responses to low nutrient concentrations, as was the case with *Gracilaria cornea* J. Agardh (Ferreira et al. 2006) and *Gracilaria birdiae* (Ursi et al. 2007). Another experiment cultivating *G. tenuifrons* under different concentrations of nitrate (NO_3^-) observed a similar growth rate of around $8\% \text{ day}^{-1}$ on samples with 150, 250, 500, and 750 μM nitrate, showing that an increase in nitrogen availability also did not influence the alga's growth (Wanderley 2009).

On top of that, the choice of culture medium for a given macroalga culture is fundamental (Ursi and Plastino 2001) and can be an important factor in the economic viability of production. Many studies have used von Stosch's (VS) solution for laboratory cultivation and a few have been carried out with *G. tenuifrons* (Torres et al. 2014; Faria 2022; Serra et al. 2024). However, when comparing the costs of enrichment with VS to the Forth used in this study, a large difference in values was observed. The price of VS solution diluted to 50% was calculated to be R\$ 0.028 per liter of growing medium. Meanwhile, Forth 50% costs only R\$ 0.003 per liter of medium, nine times less than VS. This may be because Forth is a common commercial orchid fertilizer, while VS is a culture media used in laboratory experiments and supplied with other components and vitamins (Table 1). Therefore, the use of Forth as an enrichment in seedling cultivation could be an advantage in reducing production costs.

According to Prasanna et al. (2007), among the various factors that influence the pigment composition of algae, environmental factors such as light and nutrients, particularly nitrogen, are the most important. *Gracilariopsis tenuifrons* showed significant differences in phycobiliprotein concentrations after cultivation, being 33% lower when Forth 50% was used. Phycoerythrin and phycocyanin are considered the main nitrogen reserves in red algae (Ribeiro et al. 2013), being biosynthesis and storage common during periods of high nitrogen availability in the medium. Similar results were described by Vergara et al. (1995) for *Gracilariopsis lemaneiformis* (Bory) E.Y. Dawson, Acleto & Foldvik, and Jones et al. (1996) for *Gracilaria edulis* (S.G. Gmelin) P.C. Silva. Chlorophyll-a and total carotenoids also showed higher concentrations in the samples with Forth 100%, but these results were not significant. In this case, lower need for nitrogen for the synthesis of these pigments can be attributed to this response (Bird et al. 1982), so they are less affected by the availability in the environment.

Nitrogen storage is an important ecological strategy that helps maintain the algae's growth during periods of low external nitrogen concentrations (Lapointe 1981). Furthermore, it is believed that species

of the genus *Gracilaria* show a greater response to changes in nutritional concentration, which can be partially attributed to their phycobilisomes, where phycobiliproteins are arranged (Jones et al. 1996). The storage of nitrogen in the form of phycobilins can help meet the nutritional demand when photosynthesis is stimulated by exposure to higher light levels. On that basis, the nitrogen storage observed in our study can explain why the increase in nutrient availability did not lead to higher growth. One possibility for increasing growth in the conditions studied in our research could be to cultivate *G. tenuifrons* in brighter conditions, in order to force the use of nitrogen sources to incorporate it to form carbon skeletons through CO₂ fixation.

Sondak et al. (2017) considered 10% as a standard value for the ratio of dry biomass to fresh biomass of marine macroalgae. However, this study found an average dry biomass ratio of 15.15% for *G. tenuifrons*, a value 50% higher than that used by Sondak et al. (2017). There were no significant differences between the treatments, so other limiting factors, such as light, may have hindered greater biomass accumulation. Serra (2013) obtained an increase in dry biomass values for *G. tenuifrons* when higher irradiance was used. On the other hand, Wanderley (2009) observed a decrease in dry biomass percentage in *G. tenuifrons* when increasing the nitrogen availability, which could be related to higher protoplast production rather than cell wall production.

As with the phycobiliproteins, the nitrogen content was significantly higher in the Forth 100% samples, which were 25% higher than in the Forth 50% samples. This may be directly related to pigment concentration since phycoerythrin and phycocyanin are the main nitrogen reserves in red algae (Ribeiro et al. 2013). Furthermore, according to Jones et al. (1996), red algae show a greater association between their elemental N content and N concentrations in the medium, compared to green and brown algae, which may be due to the high N storage in this group. Ryther et al. (1981) doubled the nitrogen content of *Gracilaria tikvahiae* McLachlan after immersion in an N-enriched medium for just 6 h, showing the rapid absorption of this compound.

Unlike nitrogen, carbon and hydrogen showed higher concentrations in the samples with Forth 50%, with only the differences for carbon being significant. Comparing these results with those of Wanderley (2009), who also studied the responses of *G. tenuifrons* under different amounts of enrichment, in this case nitrate, a higher elemental composition of carbon and hydrogen was observed in the media with lower nitrate availability. These results may be related to the C/N ratio, in which a higher concentration of carbon is observed in macroalgae in situations of lower nitrogen availability in the environment (Pinchetti et al. 1998). In addition, as the carbon content of macroalgae can be affected by various environmental conditions (Quan et al. 2014), other factors could be modified to increase the uptake of this compound such as light, which would increase photosynthetic activity by using the available nitrogen to produce carbon structures.

Finally, concerning CO₂ sequestration, Table 2 compares the sequestration of *G. tenuifrons* with that of other species, some of which have great ecological and economic value (Sondak et al. 2017). These include *Saccharina* and *Kappaphycus*, the two most aquaculture seaweed-produced groups in the world

(FAO 2021). From the data, under the conditions of this study, *G. tenuifrons* showed a similar sequestration to other groups of red seaweeds such as *Gracilaria*, *Pyropia*, and *Kappaphycus* and brown seaweeds like *Saccharina* and *Undaria*, which demonstrates its potential for production and carbon fixation. However, these data were calculated considering the same proportion of dry biomass (15%), so knowing the specific value for each group is important in order to be able to compare data that is more in line with reality. Furthermore, these amounts can vary depending on species, seasons, and local, due to variations in environmental conditions and cultivation parameters (Hasselström and Thomas 2022). Therefore, searching for the best environmental conditions for each algae cultivation is crucial to increase yield and quality.

In conclusion, our study provides important insights concerning the use of the low-cost commercial fertilizer Forth (30-10-10), which proved to be an efficient and economical enrichment option for indoor seaweed production. However, our results suggest that increased enrichment concentration (50% versus 100%) did not further enhance growth, indicating the existence of other environmental limitations, such as light availability. Nevertheless, the higher pigment content in the Forth 100% samples suggest an increase in nitrogen storage, partly explaining the no difference in growth rate but highlighting the potential for optimizing nutrient utilization in cultivation practices. Additionally, our results demonstrate the potential of *G. tenuifrons* as a promising biomass producer with efficient CO₂ sequestration levels for the conditions tested.

Our study also showed good results for seedling production by clonal propagation of *G. tenuifrons* using Forth 30-10-10, but further experiments focusing on optimizing growth conditions, particularly by increasing luminosity, are necessary to enhance the photosynthetic activity leading to a higher biomass production through nutrient utilization and carbon fixation. Moreover, other studies should be carried out to analyze the feasibility of Forth for seedling production through reproductive cells, since it includes many economically important species.

Our findings also reveal that *G. tenuifrons* exhibits a CO₂ sequestration potential comparable to other macroalgal species reported in the literature. The method used for calculating CO₂ sequestration can be used for any species given its carbon content and dry biomass ratio, being relevant for large-scale cultivations that wish to account for its carbon fixation and participate in carbon credit markets. Thus, considering its growth and CO₂ sequestration capabilities, *G. tenuifrons* presents itself as a good option for cultivation in Brazil.

Declarations

Competing Interests:

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author's contributions

MEOP and FC conceived the study and designed the experiments, while MEOP was responsible for conducting the experiments, analyzing the data, and performing the statistical analyses. MEOP wrote the initial draft of the manuscript and FC performed editing and revisions. FC provided analytical and statistical advisor and financial support for the study. All authors have read, contributed, and approved the final version of the manuscript.

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References

1. Alemañ AE, Robledo D, Hayashi L (2019) Development of seaweed cultivation in Latin America: current trends and future prospects. *Phycologia* 58(5):462–471
2. Arbaiza S, Avila-Peltroche J, Castañeda-Franco M, Mires-Reyes A, Advíncula O, Baltazar P (2023) Vegetative propagation of the commercial red seaweed *Chondracanthus chamissoi* in Peru by secondary attachment disc during indoor cultivation. *Plants* 12:1940
3. Barbosa-Silva MS, Borburema HDS, Fernandes FO, Nóbrega MF, Marinho-Soriano E (2023) Projecting environmental suitability areas for the seaweed *Gracilaria birdiae* (Rhodophyta) in Brazil: Implications for the aquaculture pertaining to five environmentally crucial parameters. *J Appl Phycol* 35:773–784
4. Beer S, Eshel A (1985) Determining phycoerythrin and phycocyanin concentration in aqueous crude extracts of red algae. *Mar Freshw Res* 36(6):785–792
5. Bird KT, Habig C, DeBusk T (1982) Nitrogen allocation and storage patterns in *Gracilaria tikvahiae* (Rhodophyta). *J Phycol* 18:344–348
6. Brito L, Silva S (2005) Fenología y ciclo de vida del alga *Gracilariopsis tenuifrons* (Gracilariaceae) en Sucre, Venezuela. *Rev Biol Trop* 53:67–73
7. Carneiro MMA, Resende JFD, Oliveira SR, Fernandes FO, Borburema HDS, Barbosa-Silva MS, Ferreira ABG, Marinho-Soriano E (2021) Performance of the agarophyte *Gracilariopsis tenuifrons* in a multi-trophic aquaculture system with *Litopenaues vannamei* using water recirculation. *J Appl Phycol* 33:481–490
8. Chung IK, Beardall J, Mehta S, Sahoo D, Stojkovic S (2011) Using marine macroalgae for carbon sequestration: a critical appraisal. *J Appl Phycol* 23:877–886
9. Duarte CM, Wu J, Xiao X, Bruhn A, Krause-Jensen D (2017) Can seaweed farming play a role in climate change mitigation and adaptation? *Front Mar Sci* 4:100

10. Duarte CM, Gattuso JP, Hancke K, Gundersen H, Filbee-Dexter K, Pedersen MF, Middelburg JJ, Burrows MT, Krumhansl KA, Wernberg T, Moore P, Pessarrodona A, Ørberg SB, Pinto IS, Assis J, Queirós AM, Smale DA, Bekkby T, Serrão EA, Krause-Jensen D (2022) Global estimates of the extent and production of macroalgal forests. *Glob Ecol Biogeogr* 31(7):1422–1439
11. Edwards P (1970) Illustrated guide of seaweeds and seagrasses in vicinity of Porto Arkansas, Texas. *Contrib Mar Sci* 15:1–228
12. Faria AVFD (2022) Estudos populacionais em *Gracilariopsis tenuifrons* (Gracilariales, Rhodophyta): aspectos fisiológicos e moleculares. Dissertation, Universidade de São Paulo
13. FAO (2021) Seaweeds and microalgae: an overview for unlocking their potential in global aquaculture development. Rome, FAO
14. Fernandes FO, Oliveira SR, Klein V, Carneiro MAA, Colepicolo P, Marinho-Soriano E (2017) Effect of fertilization pulses on the production of *Gracilaria birdiae* seedlings under laboratory and field conditions. *J Appl Phycol* 29:695–705
15. Ferreira LB, Barufi JB, Plastino EM (2006) Growth of red and green strains of the tropical agarophyte *Gracilaria cornea* J. Agardh (Gracilariales, Rhodophyta) in laboratory. *Revta Brasil Bot* 29(1):187–192
16. Gupta V, Trivedi N, Simoni S, Reddy CRK (2018) Marine macroalgal nursery: A model for sustainable production of seedlings for large scale farming. *Algal Res* 31:463–468
17. Hasselström L, Thomas JBE (2022) A critical review of the life cycle climate impact in seaweed value chains to support carbon accounting and blue carbon financing. *Clean Environ Syst* 6:100093
18. Hernández I, Pérez-Pastor A, Vergara JJ, Martínez-Aragón JF, Fernández-Engo MA, Pérez-Lloréns JL (2006) Studies on the biofiltration capacity of *Gracilariopsis longissima*: from microscale to macroscale. *Aquaculture* 252(1):43–53
19. Jaiswar S, Dawange PS, Thanth C, Mantri VA (2021) Apical, sub-apical, and basal explants of industrially exploited marine red alga *Gracilaria salicornia* exhibited differential response to commercial seaweed-derived plant bio-stimulants. *J Appl Phycol* 33:3975–3985
20. Jeffrey SW, Humphrey GF (1975) New spectrophotometric equations for determining chlorophylls a, b, c1 and c2 in higher plants, algae and natural phytoplankton. *Biochem Physiol Pflanzen* 167(2):191–194
21. Jiksing C, Ongkudon MM, Thien VY, Rodrigues KF, Yong WTL (2022) Recent advances in seaweed seedling production: a review of eucheumatoids and other valuable seaweeds. *Algae* 37(2):105–121
22. Jones AB, Dennison WC, Stewart GR (1996) Macroalgal response to nitrogen source and availability: amino acid metabolic profiling as a bioindicator using *Gracilaria edulis* (Rhodophyta). *J Phycol* 32:757–766
23. Krause-Jensen D, Lavery P, Serrano O, Marbà N, Masque P, Duarte CM (2018) Sequestration of macroalgal carbon: the elephant in the Blue Carbon room. *Biol Lett* 14(6):20180236
24. Lapointe BE (1981) The effects of light and nitrogen on growth, pigment content, and biochemical composition of *Gracilaria foliifera* v. *angustissima* (Gigartinales, Rhodophyta). *J Phycol* 17:90–95

25. Lignell A, Pedersén M (1989) Effects of pH and inorganic carbon concentration on growth of *Gracilaria secundata*. Brit Phycol J 24(1):83–89
26. Marinho-Soriano E (2017) Historical context of commercial exploitation of seaweeds in Brazil. J Appl Phycol 29:665–671
27. Murakami EA, Rodrigues L (2009) Resposta das algas perifíticas às alterações de temperatura e ao enriquecimento artificial de nutrientes em curto período de tempo. Acta Scientiarum Biol Sci 31(3):273–284
28. Naik KK, Naik TP (2020) Seaweed culture – a brief review. J Environ Biotech Micro 1(2):6–10
29. Oliveira EC (1998) The seaweed resources of Brazil. In: Critchley T, Ohno M (eds) Seaweed resources of the world. JICA, Yokosuka, pp 366–371
30. Oliveira EC, Horta PA, Amancio CE, Sant’anna CL (2002) Algas e angiospermas marinhas bênticas do litoral brasileiro: diversidade, exploração e conservação. In: Workshop sobre avaliação e ações prioritárias para a conservação da biodiversidade das zonas costeira e marinha. Relatório Técnico, Brasília, Ministério do Meio Ambiente 411–416
31. Pedersen MF, Bórum J (1996) Nutrient control of algal growth in estuarine waters. Nutrient limitation and the importance of nitrogen requirements and nitrogen storage among phytoplankton and species of macroalgae. Mar Ecol Prog Ser 142:261–272
32. Pendleton L, Donato DC, Murray BC, Crooks S, Jenkins WA, Sifleet S, Craft C, Fourqurean JW, Kauffman JB, Marbà N, Megonigal P, Pidgeon E, Herr D, Gordon D, Baldera A (2012) Estimating global blue carbon emissions from conversion and degradation of vegetated coastal ecosystems. PLoS ONE 7(9):43542
33. Pinchetti JLG, Fernández EC, Díez PM, Reina GG (1998) Nitrogen availability influences the biochemical composition and photosynthesis of tank-cultivated *Ulva rigida* (Chlorophyta). J Appl Phycol 10:383–389
34. Plastino EM (1991) Cultivo *in vitro*, estudos reprodutivos e biossistemática de algas gracilarióides (Rhodophyta, Gracilariales) de talo cilíndrico. Dissertation, Universidade de São Paulo
35. Porse H, Rudolph B (2017) The seaweed hydrocolloid industry: 2016 updates, requirements, and outlook. J Appl Phycol 29(5):2187–2200
36. Prasanna R, Sood A, Suresh A, Nayak S, Kaushik BD (2007) Potentials and applications of algal pigments in biology and industry. Acta Bot Hung 49(1–2):131–156
37. Radulovich R, Neori A, Valderrama D, Reddy CRK, Cronin H, Forster J (2015) Farming of seaweeds. In: Tiwari BK, Troy DJ (eds) Seaweed sustainability – Food and non-food applications. Academic, MA, USA, pp 27–59
38. Ribeiro ALNL, Tesima KE, Souza JMC, Yokoya NS (2013) Effects of nitrogen and phosphorus availabilities on growth, pigment, and protein contents in *Hypnea cervicornis* J. Agardh (Gigartinales, Rhodophyta). J Appl Phycol 25:1151–1157
39. Ryther JH, Corwin N, DeBusk TA, Williams LD (1981) Nitrogen uptake and storage by the red alga *Gracilaria tikvahiae* (McLachlan, 1979). Aquaculture 26:107–115

40. Saminathan KR, Ashok KS, Veeragurunathan V, Mantri VA (2015) Seedling production in the industrially important agarophyte *Gracilaria dura* (Gracilariales, Rhodophyta). J Appl Phycol 27:1541–1548
41. Serra DR (2013) Resposta de *Gracilariopsis tenuifrons* (Gracilariales – Rhodophyta) a estímulos de irradiância *in vitro*. Dissertation, Universidade de São Paulo
42. Serra DR, Floh EIS, Chow F (2024) Metabolic changes of the red marine alga *Gracilariopsis tenuifrons* elicited by high PAR in laboratory. Br J Bot. <https://doi.org/10.1007/s40415-024-01015-w>
43. Spillias S, Valin H, Batka M, Sperling F, Havlík P, Leclère D, Cottrell RS, O'Brien KR, McDonald-Madden E (2023) Reducing global land-use pressures with seaweed farming. Nat Sustain 6(4):380–390
44. Sondak CFA, Ang PO Jr, Beardall J, Bellgrove A, Boo SM, Gerung GS, Hepburn CD, Hong DD, Hu Z, Kawai H, Largo D, Lee JA, Lim P-E, Mayakun J, Nelson WA, Oak JH, Phang S-M, Sahoo D, Peerapornpis Y, Yang Y, Chung IK (2017) Carbon dioxide mitigation potential of seaweed aquaculture beds (SABs). J Appl Phycol 29:2363–2373
45. Torres PB, Chow F, Santos DYAC (2014) Growth and photosynthetic pigments of *Gracilariopsis tenuifrons* (Rhodophyta, Gracilariaceae) under high light in vitro culture. J Appl Phycol 27:1243–1251
46. Torres P, Santos JP, Chow F, Santos DYAC (2019) A comprehensive review of traditional uses, bioactivity potential, and chemical diversity of the genus *Gracilaria* (Gracilariales, Rhodophyta). Algal Res 37:288–306
47. Ursi S, Plastino EM (2001) Crescimento *in vitro* de linhagens de coloração vermelha e verde clara de *Gracilaria birdiae* (Gracilariales, Rhodophyta) em dois meios de cultura: análise de diferentes estágios reprodutivos. Revta Brasil Bot 24(4):587–594
48. Ursi S, Guimarães M, Plastino EM (2008) Deleterious effect of TRIS buffer on growth rates and pigment content of *Gracilaria birdiae* Plastino & E.C. Oliveira (Gracilariales, Rhodophyta). Acta Bot Bras 22(3):891–896
49. Vergara JJ, Bird KT, Niell FX (1995) Nitrogen assimilation following NH_4^+ pulses in the red alga *Gracilariopsis lemaneiformis*: effect on C metabolism. Mar Ecol Prog Ser 122:253–263
50. Wanderley A (2009) Influência da disponibilidade de nitrato sobre crescimento, atividade da nitrato redutase, composição química e captação de nitrato e fosfato em *Gracilariopsis tenuifrons* (Gracilariales, Rhodophyta). Dissertation, Universidade de São Paulo
51. Quan W, Ying M, Kang H, Xu C, Zhou Q, Liang W, Lin Z, Cai J (2014) Marine algae culture and the estimation of carbon sink capacity in the coastal areas of China. J Fish China 38(4):515–521
52. Wellburn AR (1994) The spectral determination of chlorophylls a and b, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. J Plant Physiol 144:307–313
53. Zhang R, Wang Q, Shen H, Yang Y, Liu P, Dong Y (2024) Environmental benefits of macroalgae products: A case study of agar based on life cycle assessment. Algal Res 78:103384

Figures

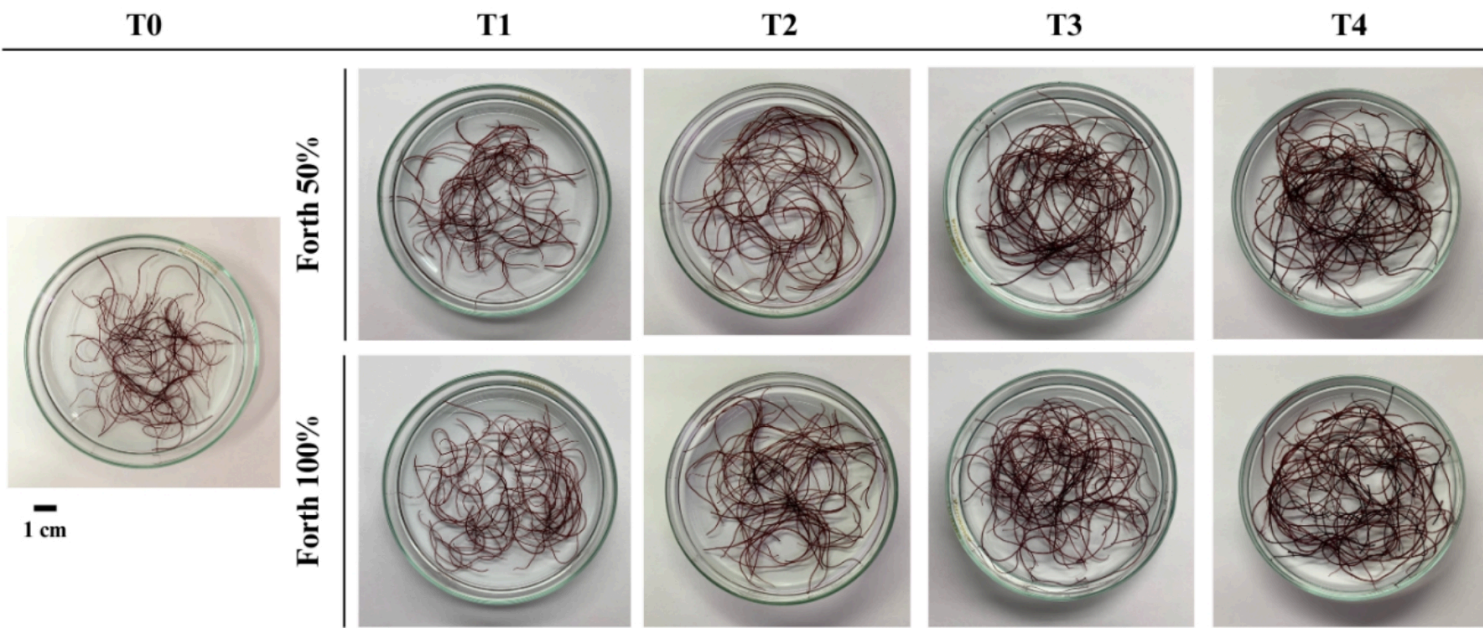


Figure 1

General and visual appearance of *Gracilariopsis tenuifrons* and the increasing biomass under both nutritional Forth treatments, Forth 50% and Forth 100%, at T0 and over the experimental period, T1-T4

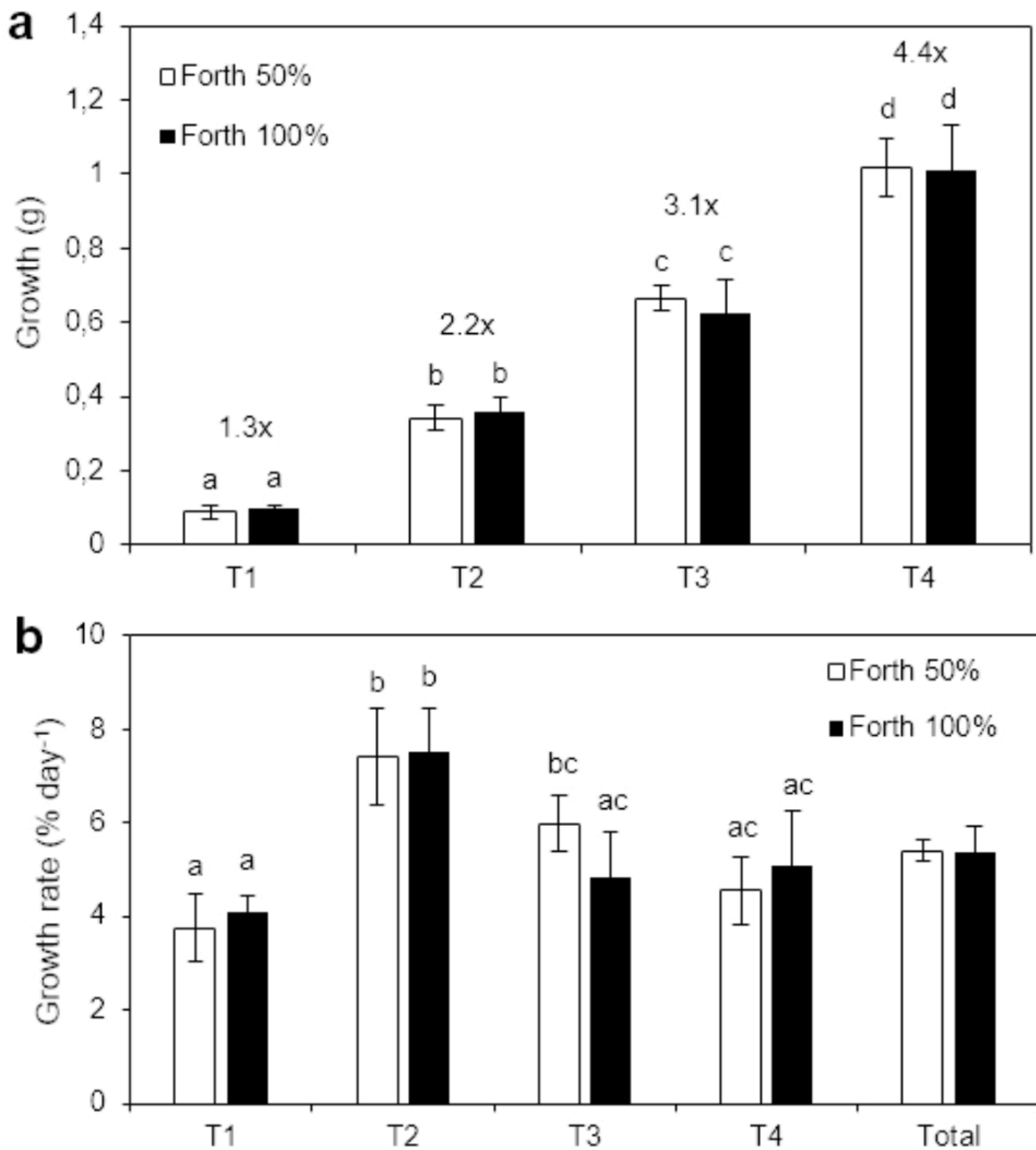


Figure 2

Physiological performance concerning **(a)** growth and duplication coefficient (representing by number with 'x') and **(b)** growth rate of *Gracilariopsis tenuifrons* under both nutritional Forth treatments, Forth 50% and Forth 100%, over the experimental period, T1-T4, and total experimental period (Total). Different letters represent significant differences ($p < 0.05$)

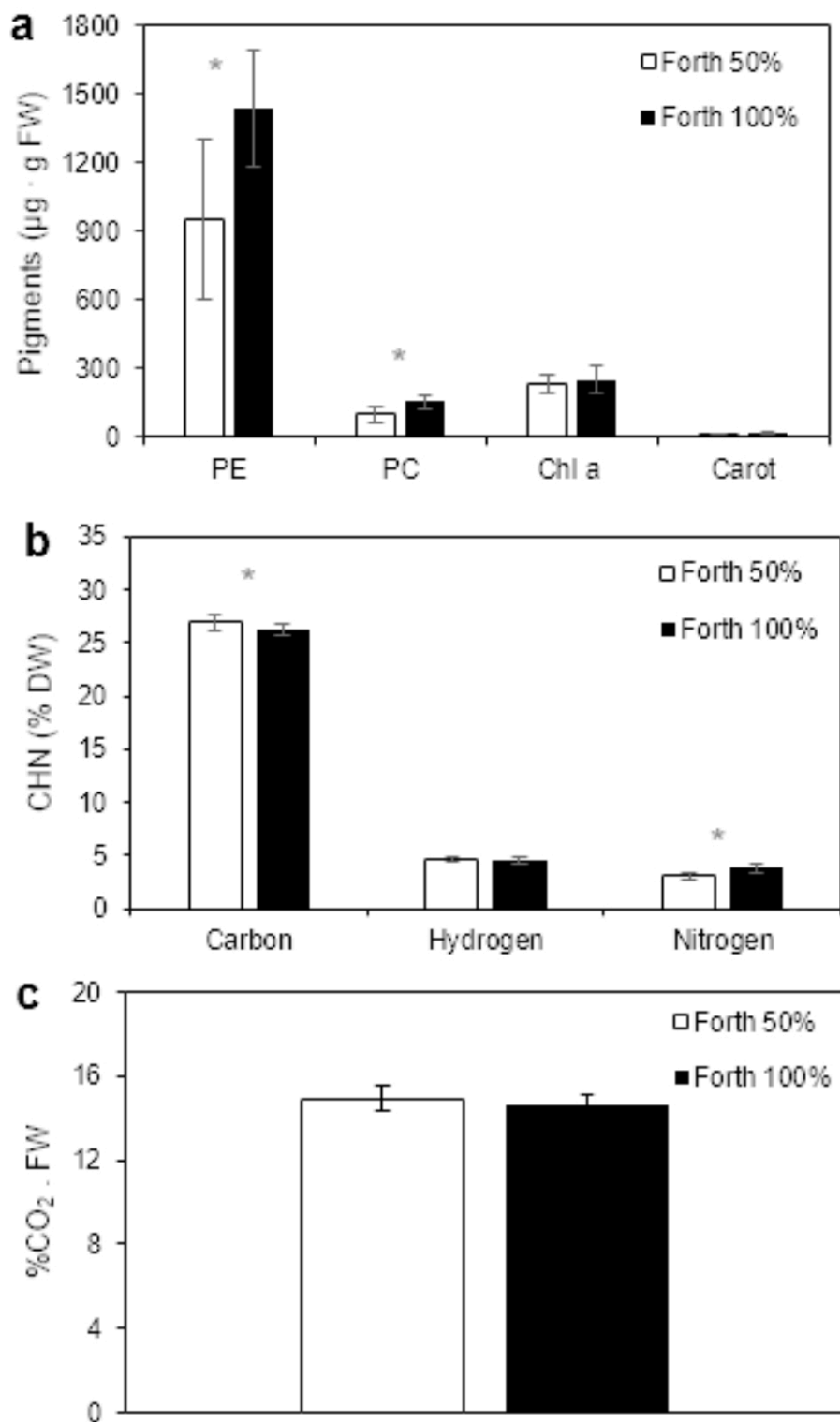


Figure 3

Chemical composition of (a) photosynthetic pigment content, (b) elemental content of carbon, hydrogen, and nitrogen (CHN), and (c) CO₂ fixation per fresh weight (FW) in samples of *Gracilariopsis tenuifrons* under both nutritional Forth treatments, Forth 50% and Forth 100%, at the total experimental period (four weeks). Asterisks represent significant differences ($p < 0.05$; t -test)