

Toward enhancing biomass growth of the red macroalga *Gracilariopsis tenuifrons*: effects of harvesting regime, stocking density and nutrient source

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Abstract

Seaweed's growth and development are influenced by multiple abiotic factors, including light, temperature, and nutrient availability. Red macroalgae store nutrients in the form of pigments such as phycoerythrin and phycocyanin, which support growth during periods of nutrient limitation. Therefore, evaluating growth performance and physiological traits is essential for improving biomass production. In Brazil, macroalgal production is constrained by the limited availability of commercial seedlings and the high cost of nutrients used in laboratory cultivation. This study investigated the biological performance of *Gracilariopsis tenuifrons* (C.J.Bird & E.C.Oliveira) Fredericq & Hommersand, a red macroalga recognized for its resilience under culture conditions. The aim was to evaluate a low-cost fertilizer as an alternative to conventional laboratory media and to identify suitable cultivation conditions through four experiments: (1) cultivation with and without biomass removal, (2) comparison of two biomass densities, (3) indoor cultivation using different fertilizer concentrations, and (4) evaluation of von Stosch (VS) medium and Forth orchid fertilizer. Higher growth rates were observed in treatments without biomass removal. Lower biomass density resulted in a growth rate of 5% day⁻¹ whereas higher density achieved 3% day⁻¹. In indoor cultivation, no differences were detected during the first two weeks; however, by the third week, the 100% Forth treatment showed superior growth. Comparisons between VS medium and Forth treatments demonstrated similar growth performance during the two weeks, while differences became more pronounced in the third week, following the order 25% > 50% > 100% Forth. Photosynthetic analyses also revealed significant differences in photochemical dissipation parameters. Overall, the results indicate that Forth is a low-cost and effective alternative for macroalgal cultivation, potentially strengthening the seaweed production chain in Brazil.

Introduction

Gracilarioid red seaweeds (Gracilariales), particularly species of *Gracilaria* and *Gracilariopsis*, have gained considerable attention for decades as a high-quality raw material for agar production, a cell-wall hydrocolloid derived from sulfated polysaccharides (Marinho-Soriano 2001; Mendes et al. 2024; Mulio et al. 2025). *Gracilaria* remains the largest volume of aquaculture seaweed world production of agar hydrocolloid and has been increasing over the years, reaching approximately 5,180.4 thousand tons (live weight) in 2020 (FAO 2022). The rheological properties of gracilarioid agar are widely demanded for numerous applications in highly processed foods, cosmetics, pharmaceuticals, nutraceuticals, therapeutic industries, biofertilizers, bioremediation, animal feeds, biotechnologies, extraction of biochemical compounds, and energy production (Tatary and Sarabandi 2025). Additionally, studies have shown various bioactive properties of sulfated polysaccharides, including antioxidants, antiviral, antitumor, and anti-inflammatory actions (Torres et al. 2019). Traditional Asian communities harvest gracilarioid species for folk medicinal purposes (Gamero-Vega et al. 2020).

Seaweed cultivation is not a modern innovation, as numerous species have traditionally farmed by coastal communities through centuries-old practices (McHugh 2003; Cai et al. 2021; Vieria et al. 2024). Among these, red seaweed represents one of the most extensively harvested from both wild stocks and

aquaculture systems. Cultivated biomass is in high growing demand due to increasing export opportunities and rising domestic consumption (Nayar and Bott 2014). Contemporary seaweed farming largely depends on seed stock derived from natural beds or vegetative cuttings from previous harvests (Yong et al. 2022). However, there is a pressing need for alternative seedling production methods, sustainable, scalable, and capable of generating large quantities of high-quality plantlets (Saminathan et al. 2014). Such approaches can reduce dependency on natural populations and mitigate the risks of overexploitation through repeated thallus reuse (Reddy et al. 2007).

In Brazil, commercial seaweed exploitation began in the 1970s, initially relying on wild seed stock collected from natural beds. Subsequently, farming practices shifted toward simple vegetative propagation, fragmenting large thalli into smaller units to serve as seedlings (Andrade et al. 2020). While effective in the short term, this extensive practice led to the overexploitation of natural populations, resulting in rapid and large-scale declines. In response, researchers have explored laboratory-based seedling propagation, followed by hatchery production and sea-based grow-out systems. This strategy not only supports the conservation of wild marine algal resources but also enables sustainable and consistent biomass production for commercial purposes (Sollest-Pitogo et al. 2020; Peixoto and Chow 2025; Rule et al. 2025).

Successful seaweed cultivation requires the optimization of multiple abiotic factors, including light, temperature, water motion, aeration, water quality, and nutrient availability (Nardelli et al. 2024). The uptake of nutrients, CO₂, and light drives the synthesis of primary and secondary metabolites, which are essential for seaweed metabolism. Cellular constituents, such as carbohydrates and proteins, are closely linked to the nitrogen status of the tissue (Macler 1986; Chopin et al. 1995; Figueroa et al. 2021; Serra et al. 2024). Marine algae, particularly red types, have the ability to store nutrients in the form of pigments, proteins, and amino acids, which they can use to support their growth during times of nutrient scarcity (Hurd et al. 2014). In addition to their ecological functions, red seaweed is a valuable source of proteins, minerals, and lipids, which confer high commercial value in various sectors, including food, pharmaceuticals, and biotechnology.

Commonly used nutrient media for laboratory seaweed cultivation, such as F/2 (Guillard 1975), Provasoli (Provasoli 1966) and von Stosch (Edward 1970), supplement baseline nutrients in seawater and generally support good algal growth. However, their high-cost limits scalability for aquaculture and routine lab use, driving research into more affordable and scalable alternatives. Commercial agricultural fertilizers have emerged as a promising alternative, offering enhanced algal growth compared to conventional enrichment media while also improving accessibility and reproducibility in cultivation practices. Booth (1986) noted that the high nitrogen, potassium, and calcium content in macroalgae highlight their potential both as fertilizer and for growth enhancement under fertilized conditions. Moreover, the use of commercial fertilizers for the laboratory cultivation of *Gracilaria* species has also shown promising results. Kim and Yarish (2014) demonstrated that commercial products such as Jack's Special (N:P:K; 21:8:18) and Miracle-Gro (N:P:K; 24:8:16) effectively replaced the von Stosch medium,

supporting similar growth rates while significantly reduces cultivation costs. Similarly, Peixoto and Chow (2025) reported elevate biomass production values under commercial NPK fertilization (30:10:10).

While lab-based seaweed cultivation has advanced, the transition from *in vitro* seedling production to offshore grow-out remains underexplored. This phase is crucial for linking high-quality propagation with scalable farming. Vegetative propagation offers key advantages, lower mortality, shorter incubation, reduced labor, and reuse of thalli, making it ideal for commercial use. Species like *Kappaphycus*, *Gracilaria*, *Gelidiella*, and *Gelidium* have shown strong proliferation via this method (Mantri et al. 2017; García-Poza et al. 2020). Direct regeneration is faster and less technically demanding, supporting its adoption by farming communities. With rapid cycles, high survival, and uniform yields, clonal propagation often outperforms spore-based systems (Vignesh et al. 2020). However, one significant limitation in vegetative propagation is the quantity of material needed. Hurtado et al. (1992) estimated that approximately 30–40% of the harvested biomass must be retained as seed stock for the next cropping cycle in *Gracilaria*. This necessitates careful selection of suitable explants for commercial farming operations. Apical tissue explants are generally preferred due to their superior growth, regeneration capacity, and survival rates (Saminathan et al. 2014), further emphasizing the importance of refining propagation techniques for suitable production.

Low-cost fertilizers offer a promising way to enhance seaweed production while reducing cultivation costs. Nitrogen supplementation, in particular, is known to significantly boost growth and productivity under suitable conditions (Lapointe and Ryther 1979). Understanding how seaweeds respond physiologically to alternative nutrient sources is key to improving biomass yields and scaling both lab and open-sea systems. Since nitrogen and phosphorus are critical for energy metabolism and macromolecule synthesis (Barbosa-Silva et al. 2023), their availability strongly influences primary productivity. However, traditional lab media are often expensive and complex, limiting their use in large-scale operations. Commercial fertilizers, by contrast, provide essential nutrients in a more accessible and cost-effective form (Fernandes et al. 2017). Nonetheless, the physiological responses of seaweed to nutrient availability are highly species-specific, as demonstrated by Murakami and Rodrigues (2009). This underscores the importance of investigating the specific nutritional requirements and tolerance of each species. Controlled laboratory experiments are a crucial first step toward characterizing these requirements. Such studies help assess physiological performance, phenotypic plasticity, stress responses, and tolerance thresholds, key parameters for optimizing cultivation protocols and ensuring long-term production success.

In this context, the present study was designed to evaluate the growth performance and physiological features of the agarophyte *Gracilariopsis tenuifrons* (C.J. Bird e E.C. Oliveira) Fredericq & Hommersand under different cultivation parameters that are decisive for biomass production, particularly by using a low-cost commercial fertilizer. Specifically, we assessed the effects of periodic biomass harvesting (thallus removal), initial stocking density, different concentrations of a commercial fertilizer supplementation, and a comparison between a traditional culture medium and commercial fertilizer. Although previous studies have examined the effects of nitrogen and phosphorus enrichment on

macroalgal growth, few have investigated the potential of agricultural fertilizer as viable nutrient sources for laboratory cultivation. By testing the performance of a widely available plant commercial fertilizer, this study aims to generate practical insights into improving *G. tenuifrons* biomass production, with potential applications for other important macroalgal species.

Material and methods

1) Biological stock material

The strain BG0039 of the red macroalga *G. tenuifrons* from the Germplasm Bank of the Laboratory of Marine Algae 'Édison José de Paula' of the Institute of Bioscience at University of Sao Paulo, Brazil, corresponding to the female gametophytic phase (haploid, n) was used for this study.

The culture growth of stock material for subsequent experiments was established at a proportion of 1 g of algae per 1 L of culture medium enriched with 50% von Stosch nutrient solution (Ursi and Plastino 2001), salinity of 32 ppm, $110 \pm 5 \mu\text{mol photon m}^{-2} \text{s}^{-1}$, $25 \pm 1^\circ\text{C}$, photoperiod of 12 h, and air bubbling at intermittent 30 min. Material maintenance and renovation of culture medium was carried out weekly. Irradiance was provided by daylight fluorescent lamps (Philips/Osram 5200 K/40 W) and determined with a LI-COR quanta meter (LI-250, USA) and LI-COR spherical sensor (LI-193, USA). Seawater was double filtered with a CUNO filter of 2 μm and 1 μm and sterilized by ionizing radiation (UV-C lamp, QUIMIS Q884 system – 21; 3.8 L min^{-1}).

2) Experimental design

We tested four different conditions of *G. tenuifrons* cultivation: (1) removal or no-removal of exceeding growing biomass, (2) density of cultivation (g /L), (3) indoor cultivation with commercial Forth fertilizer, and (4) von Stosch nutrient solution and commercial Forth fertilizer (Fig. 1).

Experiment with removal or no-removal of exceeding biomass. Apical sections of 1 cm were grown for four weeks in concentrations of 50% and 100% of VS nutrient solution at a proportion of 5 g of algae per 1 L of cultivation medium, without and with removing excess of biomass weekly over four weeks (Fig. 1a). In total, there were four treatments ($n = 3$): VS 50% without removing biomass, VS 50% with removing biomass, VS 100% without removing biomass, and VS 100% with removing biomass. The remaining cultivation conditions were the same as those described in stock material cultivation. Growth and growth rate were assessed weekly.

Experiment of density of cultivation biomass. Two biomass densities, approximately 8 cm thallus length, 1 g L^{-1} and 4 g L^{-1} , were tested in cultivation ($n = 3$) for three weeks, both enriched with VS 50% nutrient solution (Fig. 1b) The remaining cultivation conditions were kept the same as those described in the stock material cultivation. Growth and growth rate were assessed weekly.

Experiment indoor cultivation with commercial Forth fertilizer. Two concentrations of the commercial fertilizer Forth® for orchid growth 30-10-10 (N-P-K; Tecnutri do Brasil, Brazil) were tested for three weeks (Fig. 1c). One equivalent to 50% of the nitrogen concentration of the VS nutrient solution (0.25 µM) and the other equivalent to 100% of the nitrogen concentration of the VS solution (0.5 µM). This commercial fertilizer was chosen due to its low commercial value and high N concentration. For this experiment, thallus apical portions of 10 cm were cultivated in an indoor system under semi-controlled conditions with natural light and photoperiod. Clear plastic boxes were used as aquariums ($n = 5$) with water flow of 6 L h^{-1} , corresponding to total seawater renewal in 2 h. Seawater flow was a closed system with double filtering by water bag filters at 10 µm and 0.5 µm (BN, Hydro) and stored in a 150 L tank. Temperature and light data were recorded using the HOBO Pendant Temperature/Light Data Logger probe (UA-002-xx). Growth and growth rate were assessed weekly.

Experiment with von Stosch nutrient solution and commercial Forth fertilizer. Comparison in algal performance was carried out between VS 25%, VS 50%, VS 100%, Forth 25%, Forth 50%, and Forth 100%, with $n = 3$ (Fig. 1d). Apical sections of 1 cm were grown for four weeks at $60 \pm 5 \mu\text{mol photon m}^{-2} \text{ s}^{-1}$ and the remaining cultivation conditions were kept the same as those described in the stock material cultivation. Growth and growth rate were assessed weekly. At the end of the experiment, the photosynthetic performance was assessed by *in vivo* PSII chlorophyll *a* fluorescence.

3) Analytical procedures

Growth rate. The growth was calculated from the difference of fresh mass (FM) and the growth rate calculated following Penniman et al. (1986) as $\text{Growth rate (\% day}^{-1}) = [(\text{Final FM} / \text{Initial FM})^{1/t} - 1] \times 100$. Additionally, the duplication coefficient was calculated as $\text{Final FM} / \text{Initial FM}$.

Photosynthetic performance. *In vivo* PSII chlorophyll *a* fluorescence was assessed by using a CF Imager fluorimeter (Technologica, UK). The maximum quantum yield (F_v/F_m) was evaluated in samples pre-acclimatized to the dark for 15 min and calculated according to Schreiber et al. (1986). The effective quantum yield ($Y(II)$) was measured in light-acclimated algae and calculated following Schreiber and Neubaer (1990). From the fluorescence data, the parameters photochemical dissipation ($Y(PSII)$), unregulated non-photochemical dissipation ($Y(NO)$) and regulated non-photochemical dissipation ($Y(NPQ)$) were also estimated (Roháček 2002). Relative electron transport rate (ETR_r) was estimated to be using P-I (photosynthesis-irradiance) curves.

4) Statistical analyses

Data was analyzed using the software STATISTICA v.10. All data were subjected to the Kolmogorov-Smirnov normality test and homoscedasticity Bartlett test within a 95% confidence interval ($p < 0.05$). One-way analysis of variance (ANOVA) was performed to verify the difference between treatment means. Then, once significance was verified between the means, the Newman-Keuls *post-hoc* test was applied for multiple comparison of significant differences ($p < 0.05$).

Results

Experiment without and with removing excess of biomass weekly

Significant differences were verified between the growth rates of the treatments without and with removing excess biomass under 100% VS ($p = 0.00$; Fig. 2b), whereas under 50% VS ($p = 0.15$; Fig. 2a) no differences were detected. At 50% VS the difference was registered in the first experimental week (Fig. 2a). Additionally, a trend towards an increase in the growth rate of treatment without removal was observed. At 100% VS the differences were registered for all experimental weeks, except in the first week (Fig. 2b). Additionally, increasing growth rates of treatment without removal were observed.

Experiment of density of cultivation biomass

Growth rates of treatments under the density of 1 g L^{-1} were significantly higher than density of 4 g L^{-1} (Fig. 3a), with values above $5\% \text{ day}^{-1}$ at 1 g L^{-1} treatment and below $3\% \text{ day}^{-1}$ at 4 g L^{-1} treatment. Significant differences were verified between the growth rates of the density treatments ($p = 0.00$). The growth rate values remained without statistical differences among the same treatment over the weeks.

Experiment indoor cultivation with commercial Forth fertilizer

Significant differences were verified among the growth rates of *G. tenuifrons* cultivated under 50% Forth and 100% Forth ($p = 0.00$), nevertheless only the growth rate at 100% Forth showed significant increase after three weeks (Fig. 3b).

Experiment with von Stosch nutrient solution and commercial Forth fertilizer

No significant differences were verified within the growth and doubling coefficient of *G. tenuifrons* cultivated under different concentrations of VS and Forth for the same experimental period (Fig. 4a). Only in the third week, significant increase was detected. VS nutrient solution was no different from Forth fertilizer at the same nutrient concentration, in which $25\% > 50\% > 100\%$. Comparing the growth rates among VS and Forth overtime few differences were detected (Fig. 4b).

Data from the fourth week was analyzed separately. Significant differences were verified among the growth and doubling coefficient of *G. tenuifrons* cultivated under different concentrations of VS and Forth ($p = 0.00$; Fig. 5a), with the trend of greater growth and doubling coefficient in Forth treatments than VS treatments. Comparing the growth rates among VS and Forth, the same trend was observed, major growth rates for Forth treatment are higher than VS treatments (Fig. 5b).

Additionally, after four weeks of experiment, photosynthetically performance was assessed. Significant differences were verified among the photochemical dissipation $Y(PSII)$ and unregulated non-photochemical dissipation $Y(NO)$ of *G. tenuifrons* cultivated under different concentrations of VS and Forth ($p = 0,05$; Fig. 6). Almost all $Y(PSII)$ from Forth treatments were higher than VS treatments, except at 100%, while the $Y(NO)$ was higher in VS treatments. The regulated non-photochemical dissipation $Y(NPQ)$ was not detected in any of the treatments.

The maximum quantum yield Fv/Fm (Fig. 7a) was equal for VS and Forth at 25%, while at 50% Forth and 100% Forth were higher against the respective VS treatment. The same trend was verified for relative ETR_{max} (Fig. 7b) and alpha photosynthetic efficiency (Fig. 7c).

Discussion

Optimizing macroalgal biomass growth requires identifying nutrient regimes that enhance photosynthesis and sustain growth. In controlled environments, such as a laboratory, or semi-controlled environments as indoor/outdoor land-based systems, this approach alleviates pressure on natural algal beds and minimizes ecological impacts, thereby facilitating the coexistence of sustainable aquaculture practices with wild populations. This is particularly relevant in efforts to curb the overexploitation of natural resources (Buchmann et al. 2017). The use of laboratory-based seedling production also represents a promising strategy to reduce the dependency on wild stocks (Peixoto and Chow 2025).

In aquaculture systems, macroalgal biomass is typically harvested at regular intervals once thalli attain marketable size. However, frequent harvesting such as on a weekly basis can disrupt physiological homeostasis, reduce specific growth rates, and ultimately diminish both biomass yield and the economic sustainability of cultivation operations (Hafting et al. 2015). In our study, we investigated the effects of harvesting frequency under varying nutrient regimes. Under 50% VS nutrient concentration, periodic biomass removal did not significantly affect growth rates. Conversely, under full-strength 100% VS nutrient supply, continuous biomass accumulation resulted in enhanced growth performance, primarily driven by increased apical development. The pronounced increase in apical branching observed under the 100% VS treatment suggests elevated meristematic activity, likely stimulated by greater nutrient availability, particularly nitrogen. This pattern aligns with evidence linking nitrogen-rich conditions to increased cell proliferation and growth in red algae (Lobban and Harrison 1997; Hurd et al. 2014), for example *Gracilaria domingensis* (Kützinger) Sonder ex Dickie (Bezerra et al. 2024), *Gracilaria cervicornis* (Turner) J. Agardh (Carneiro et al. 2011), *Gracilaria vermiculophylla* (Ohmi) Papenfuss (Pedersen and Johnsen, 2017), *Gracilariopsis lemaneiformis* (Bory) E.Y.Dawson, Acleto and Foldvik (Li et al. 2022), and *Gracilariopsis tenuifrons* (Peixoto and Chow 2025).

Biomass density is another critical parameter, influencing both nutrient and light availability within cultivation systems. In our study, cultures initiated at lower stocking densities ($1 \text{ g} \cdot \text{L}^{-1}$) exhibited significantly higher specific growth rates than those initiated at higher densities ($4 \text{ g} \cdot \text{L}^{-1}$). This observation corroborates earlier findings that reduced biomass loads improve nutrient accessibility

(Hurtado et al. 2008; Carton-Kawagoshi et al. 2014; Rula et al. 2021) and reduce self-shading, which otherwise compromises photosynthetic efficiency (Hernandez et al. 2005; Copertino et al. 2009). Reduced light availability within dense cultures further constrains photosynthesis, compounding the limitations imposed by nutrient diffusion (Hurd 2000; Koch 1993). These combined effects underscore the necessity of optimizing biomass loading to ensure maximum light capture and nutrient uptake. Given the pivotal role of nitrogen in supporting protein synthesis and cellular proliferation in red macroalgae (Lobban and Harrison 1994) its limited availability under high-density conditions can become a major bottleneck for growth (Hurd et al. 2014). Therefore, maintaining an appropriate balance between biomass density, nutrient supply, and light availability is essential to sustain high productivity and metabolic efficiency. Optimizing this balance also supports carbon metabolism and energy allocation, which are central to the development of robust, scalable, and economically viable macroalgal cultivation systems.

To investigate the influence of nutrient availability, we evaluated algal growth under different dilutions of VS medium and a commercial fertilizer (Forth 30-10-10), using nitrogen as the comparative element due to its pivotal role in metabolic pathways, including protein biosynthesis and pigment production (Hurd et al. 2014). Both media provide nitrogen primarily in the form of nitrate, which is readily assimilated and preferentially utilized by red algae (Naldi and Wheeler 1999). While traditional enrichment media such as PES (Provasoli Enriched Seawater) and VS remain standard and widely used in phycological research (Ursi and Plastino 2001), their high production cost represents a significant constraint for scaling up biomass and seedling production. In this context, commercial agricultural fertilizers represent promising cost-effective alternative for sustainable and scalable macroalgal cultivation (Kim and Yarish 2014; Peixoto and Chow 2025). In our study, *G. tenuifrons* achieved growth rates ranging from $13\text{--}15\%\cdot\text{day}^{-1}$ in VS treatment and $15\text{--}18\%\cdot\text{day}^{-1}$ in Forth fertilizer treatments. These rates exceed those reported for other Gracilariaceae species such as *G. lemaneiformis* (Bory) ($9.64\%\cdot\text{day}^{-1}$; Duan 2019), *G. tikvahiae* McLachlan ($8.4\%\cdot\text{day}^{-1}$; Kim and Yarish 2014), and *Gracilariopsis longissima* (S.G.Gmelin) Steentoft, L.M. Irvine & Farnham ($3.08\text{--}4.72\%\cdot\text{day}^{-1}$; Sobuj et al. 2023), underscoring the high growth potential of *G. tenuifrons* under optimized nutrient regimes. Among the treatments, Forth at 50% concentration yielded rates comparable to, or even surpassing, those observed in VS medium, confirming its viability as a low-cost substitute. After three weeks of cultivation, biomass increased up to 30-fold across most treatments, with particularly high yield observed in Forth 25% and VS 25%, where biomass increased by 35- to 37-fold. These results demonstrate the efficacy of diluted fertilizer-based media in sustaining robust biomass accumulation, which is critical for commercial-scale applications. Another study using Basfoliar® Aktiv in *Gracilaria chilensis* C.J. Bird, McLachlan & E.C. Oliveira demonstrated the suitability of this commercial fertilizer for biomass production. However, lower growth rates were reported, ranging from $0.35\text{--}4.66\%\cdot\text{day}^{-1}$ (Caroca-Valencia et al. 2023).

No visible pigment degradation was observed across treatments, indicating stable phycobiliprotein content. These light-harvesting pigments often serve not only in photosynthetic energy capture but also as nitrogen storage pools under nutrient-replete conditions (Kosovel and Talarico 1979). Chlorophyll

fluorescence measurement revealed Fv/Fm values ranging from 0.500 and 0.600, which are consistent with those typically reported for healthy red macroalgae. For instance, Usandizaga (2018) reported Fv/Fm values between 0.630 and 0.670 for *G. chilensis*, further supporting the reliability of this physiological indicator. Interestingly, the Forth 25% treatment showed the highest non-photochemical quenching (NPQ), suggesting the activation of photoprotective mechanisms that dissipate excess excitation energy as heat. This response is often associated with physiological acclimation that enables sustained growth under variable light or nutrient conditions (Genty et al. 1989; Usandizaga 2018). The absence of detectable Y(NPQ), combined with higher Fv/Fm relative to Y(II), points to low levels of photooxidative stress and efficient utilization of absorbed light energy without sign of photoinhibition.

These findings collectively indicate that *G. tenuifrons* maintained photosynthetic competence across all treatments, even under reduced nutrient concentrations, and suggest that the Forth 25% fertilizer supports not only high growth rates but also favorable physiological status.

The cultivation of *G. tenuifrons* under controlled laboratory conditions using Forth 30-10-10 at 50% concentration proved to be both biologically effective and economically viable. Growth rates achieved in Forth treatments were comparable to, or exceeded, those obtained with the conventional VS medium, while higher concentrations of Forth did not confer additional benefits. Thus, these results suggest that 50% represents an optimal threshold for maximizing growth while minimizing nutrient excess and potential environmental discharge. Consequently, the low-cost Forth 30-10-10 fertilizer at this concentration emerges as a promising low-cost alternative for biomass production in laboratory cultivation of *G. tenuifrons*. Despite the favorable outcomes, our findings also highlight the importance of maintaining low culture densities and avoiding frequent biomass removal to support optimal seedling development. These factors, when appropriately managed, can enhance both growth performance and resource-use efficiency. Nevertheless, further investigations under diverse environmental and operational conditions are essential to validate the scalability, consistency, and long-term viability of this cultivation strategy.

Enhancing biomass production under controlled conditions is of increasing interest to seaweed producers, particularly as natural algal beds face mounting pressure from overharvesting. In Brazil, decades of unsustainable exploitation of wild algal stocks have led to critical depletion on natural *Gracilaria* stocks, rendering traditional unfeasibility and calling for urgent restoration initiatives based on seedling propagation and *ex situ* cultivation.

Overall, this study highlights the strategic importance of nutrient source optimization in macroalgal aquaculture. By balancing input cost with biomass productivity, commercial fertilizers such as Forth 30-10-10 can support economically viable and environmentally sustainable aquaculture practices. Future research should explore the long-term physiological responses to fertilizer-based media, including biochemical composition and abundance. Additionally, it contributes to the advancement of sustainable aquaculture practices by demonstrating that commercially available fertilizers can serve as low cost-effective nutrient sources for macroalgal cultivation. As natural *Gracilaria* beds in Brazil continue to

decline (Oliveira et al. 2020), the establishment of efficient indoor propagation protocols becomes imperative. Our findings provide a foundational framework for supporting seedling production and biomass expansion with reduced dependency on wild populations, aligning with broader environmental conservation and resource management goals.

Future studies should prioritize long-term cultivation trials to assess the stability and reproducibility of biomass yields over extended periods. In addition, evaluating the effects of different fertilizer formulations on the biochemical profile of *G. tenuifrons*, including polysaccharides, pigments, and bioactive compounds, is essential to ensure the quality of biomass for various biotechnological applications. Finally, particular attentions should be given to the potential ecological impacts of nutrient discharge from large-scale cultivation systems, including eutrophication risks and interaction with surrounding marine ecosystems. Addressing these aspects will be critical for developing environmentally responsible and commercially scalable macroalgal aquaculture practices.

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Table

Table 1 Two-way ANOVA table for growth, duplication coefficient and growth rate of treatments grown in von Stosch and Forth on weeks 1, 2 and 3. SQ: sums of squares, DF: degrees of freedom, MS: mean

squares, F: Fischer value, *p*: probability

Growth					
	SQ	GL	MS	F	<i>p</i>
Intercept	210481.50	1	210481.50	893.51	0.00
Treatment	3201.30	5	640.30	2.72	0.03
Days	177045.90	2	88523.00	375.78	0.00
Time	7917.00	10	791.70	3.36	0.00
Error	8480.40	36	235.60		
Doubling coefficient					
Intercept	9821.80	1	9821.80	1042.36	0.00
Treatment	128.05	5	25.61	2.72	0.03
Days	7081.84	2	3540.92	375.78	0.00
Time	316.68	10	31.67	3.36	0.00
Error	339.22	36	9.42		
Growth rate					
Intercept	15006.97	1	15006.97	10473.92	0.00
Treatment	23.39	5	4.68	3.26	0.01
Days	15.93	2	7.97	5.56	0.01
Time	63.60	10	6.36	4.44	0.00
Error	51.58	36	1.43		

Figures

a) Experiment with removal or no-removal of exceeding biomass



- $n = 3$
- Nutrient solution: von Stosch
- $100 \pm 5 \mu\text{mol f\acute{o}ton m}^{-2} \text{s}^{-1}$
- Growth rate
- Growth



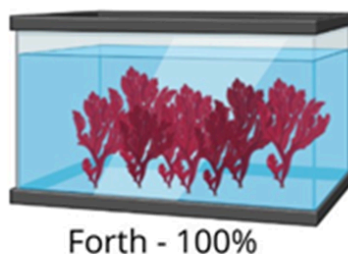
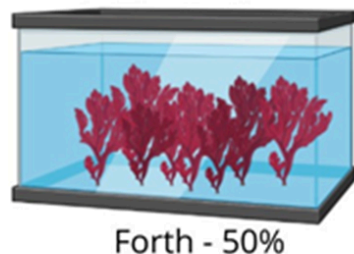
b) Experiment of density of cultivation biomass



- $n = 5$
- Nutrient solution: von Stosch
- $100 \pm 5 \mu\text{mol f\acute{o}ton m}^{-2} \text{s}^{-1}$
- Growth rate
- Growth



c) Experiment indoor cultivation with commercial Forth fertilizer



- $n = 5$
- Growth rate
- Growth



d) Experiment with von Stosch nutrient solution and commercial Forth fertilizer

Forth - 25%



von Stosch- 25%



Forth - 50%



von Stosch - 50%



Forth - 100%



von Stosch- 100%



- $n = 3$
- $60 \pm 5 \mu\text{mol f\acute{o}ton m}^{-2} \text{s}^{-1}$
- Growth rate
- Growth
- Duplication coefficient
- Photosynthesis



Figure 1

General experimental design of the four performed experiments, including treatments and laboratorial. (a) Experiment without and with weekly excess biomass removal. (b) Experiment of density of cultivation biomass. (c) Experiment indoor cultivation with commercial Forth fertilizer. (d) Experiment comparing von Stosch nutrient solution and commercial Forth fertilizer. Created with BioRender.com

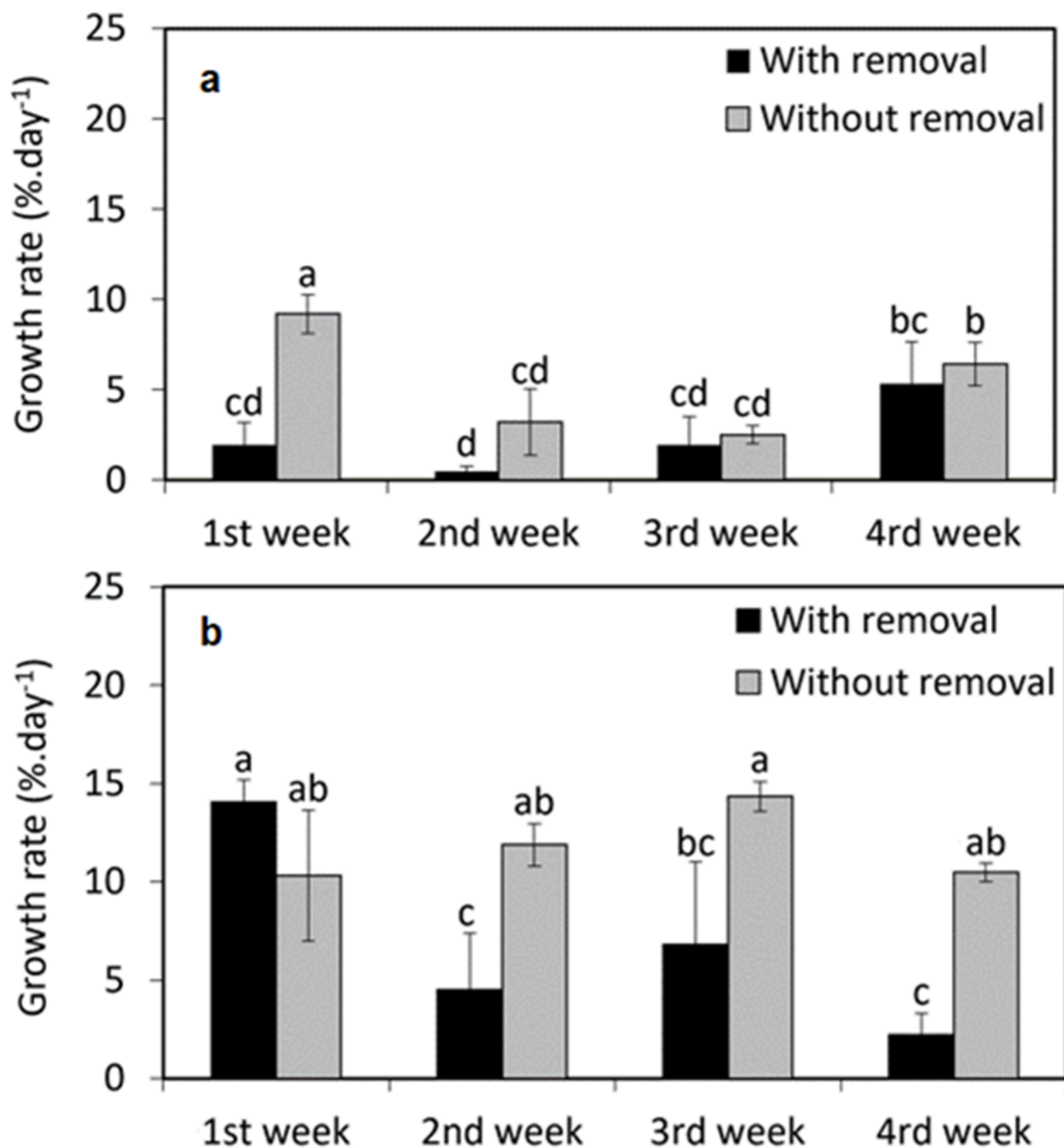


Figure 2

Growth rate (% day⁻¹) of *Gracilariopsis tenuifrons* grown in von Stosch at concentrations of (a) 50% and (b) 100%, with and without excess biomass removal over four weeks. Values represent the mean and standard deviation ($n = 3$). Letters represent statistical differences ($p < 0.05$)

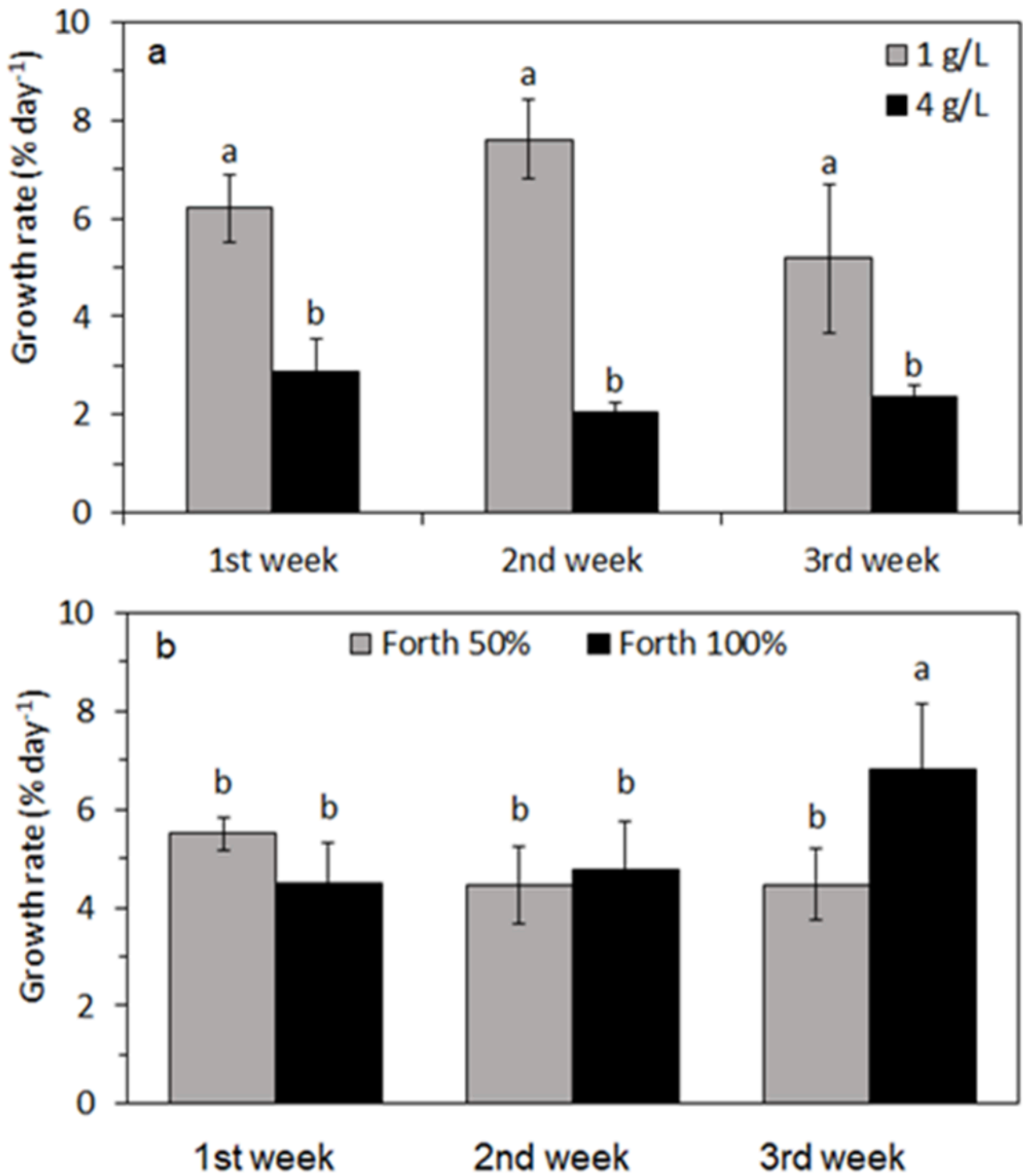


Figure 3

Growth rate (% day⁻¹) of *Gracilariopsis tenuifrons* grown at (a) density of 1 g mL⁻¹ and 4 g mL⁻¹ and (b) Forth at concentrations of 50% and 100%, over three weeks without excess biomass removal. Values represent the mean and standard deviation ($n = 3$). Letters represent statistical differences ($p < 0.05$)

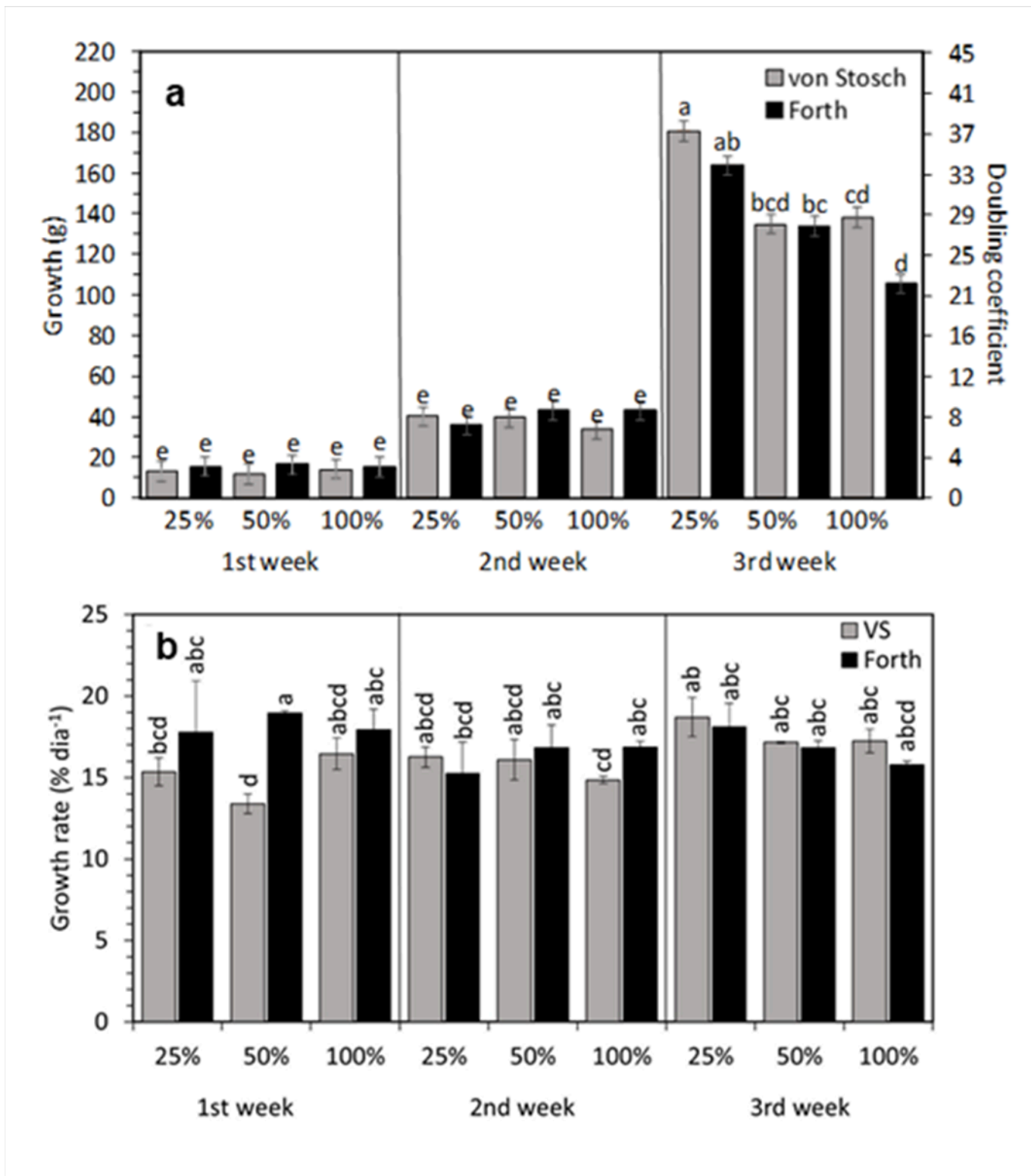


Figure 4

(a) Growth (g) and doubling coefficient and (b) growth rate (% day⁻¹) of *Gracilariopsis tenuifrons* in von Stosch (VS) and Forth (FT) treatments at concentrations of 25%, 50% and 100%, over three weeks. Values represent the mean and standard deviation ($n = 3$). Letters represent statistically differences within each treatment ($p < 0.05$)

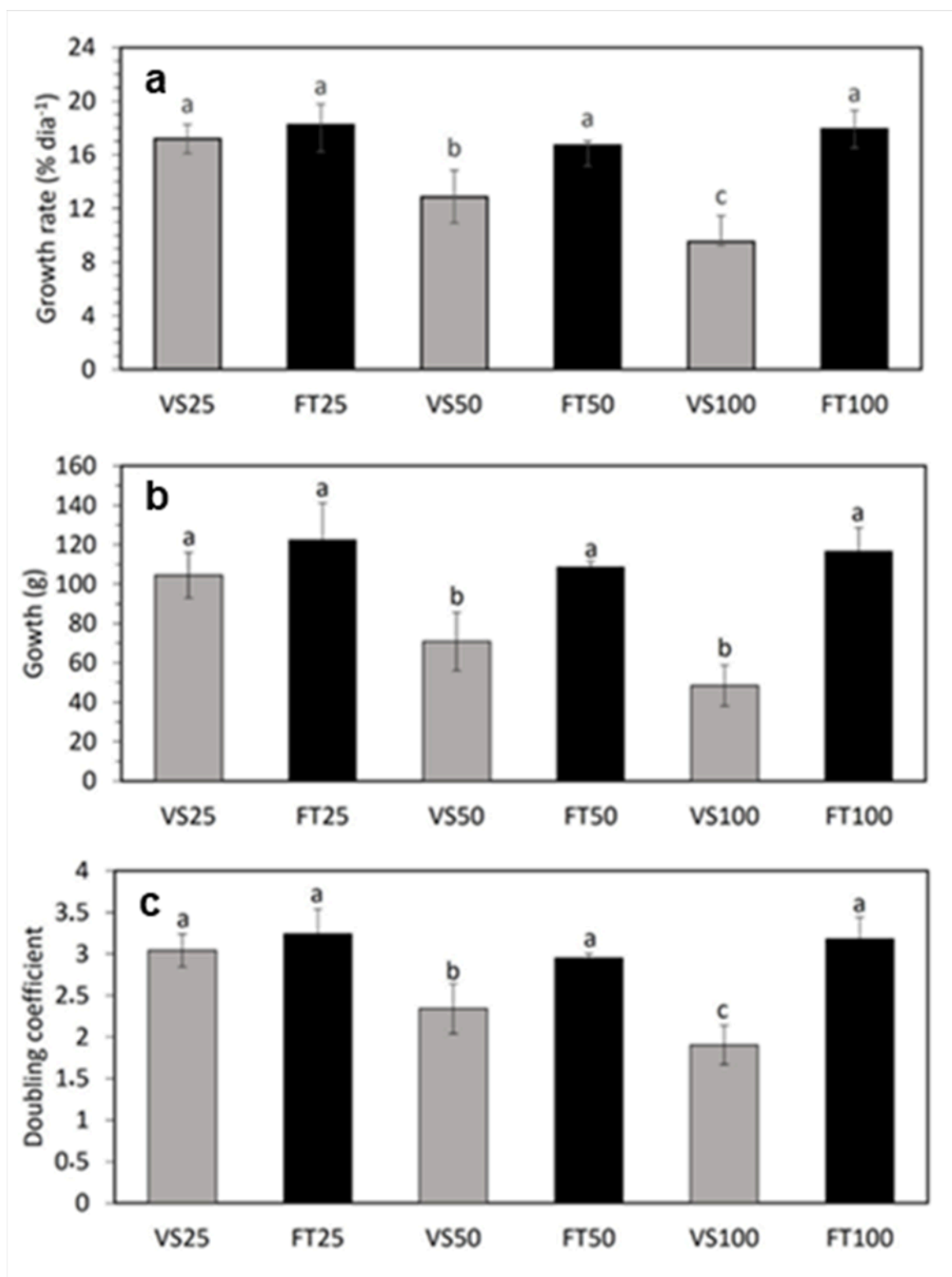


Figure 5

(a) Growth rate (% day⁻¹), (b) Growth (g), and (c) Doubling coefficient of *Gracilariopsis tenuifrons* under von Stosch (VS) and Forth (FT) treatments at concentrations of 25%, 50%, and 100% after four weeks. Values represent the mean ± standard deviation (n = 5). Letters indicate statistically significant differences (p < 0.05), with comparisons made within each treatment group

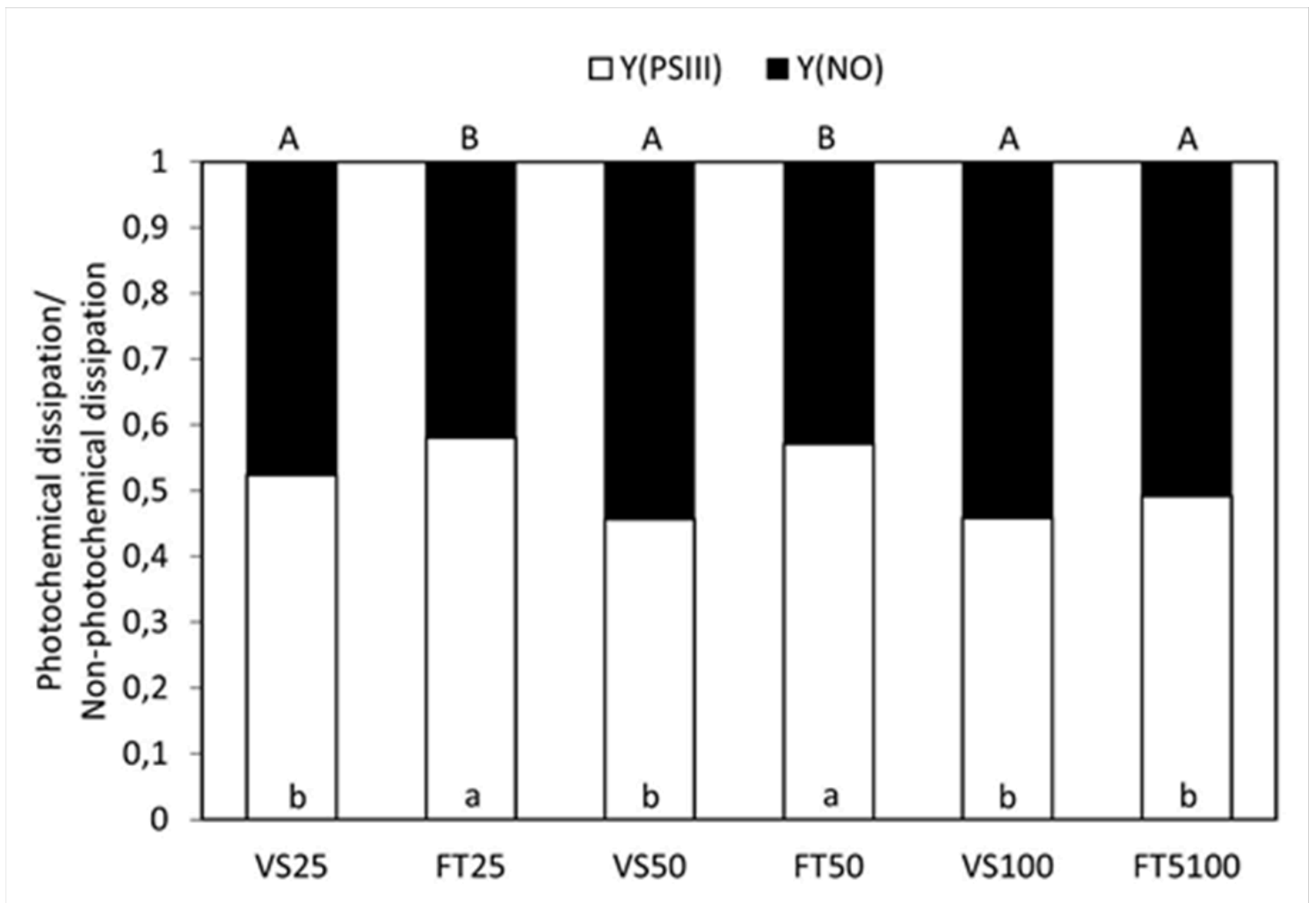


Figure 6

Photochemical dissipation [Y(PSII)] and non-regulated non-photochemical dissipation [Y(NO)] of *Gracilariopsis tenuifrons* in von Stosch (VS) and Forth (FT) treatments at concentrations of 25%, 50% and 100% measured in week four after biomass removal. Values represent the mean ($n= 5$) with statistically differences at lowercase letters for Y(NO) and uppercase letters for Y(PSII) ($p < 0.05$)

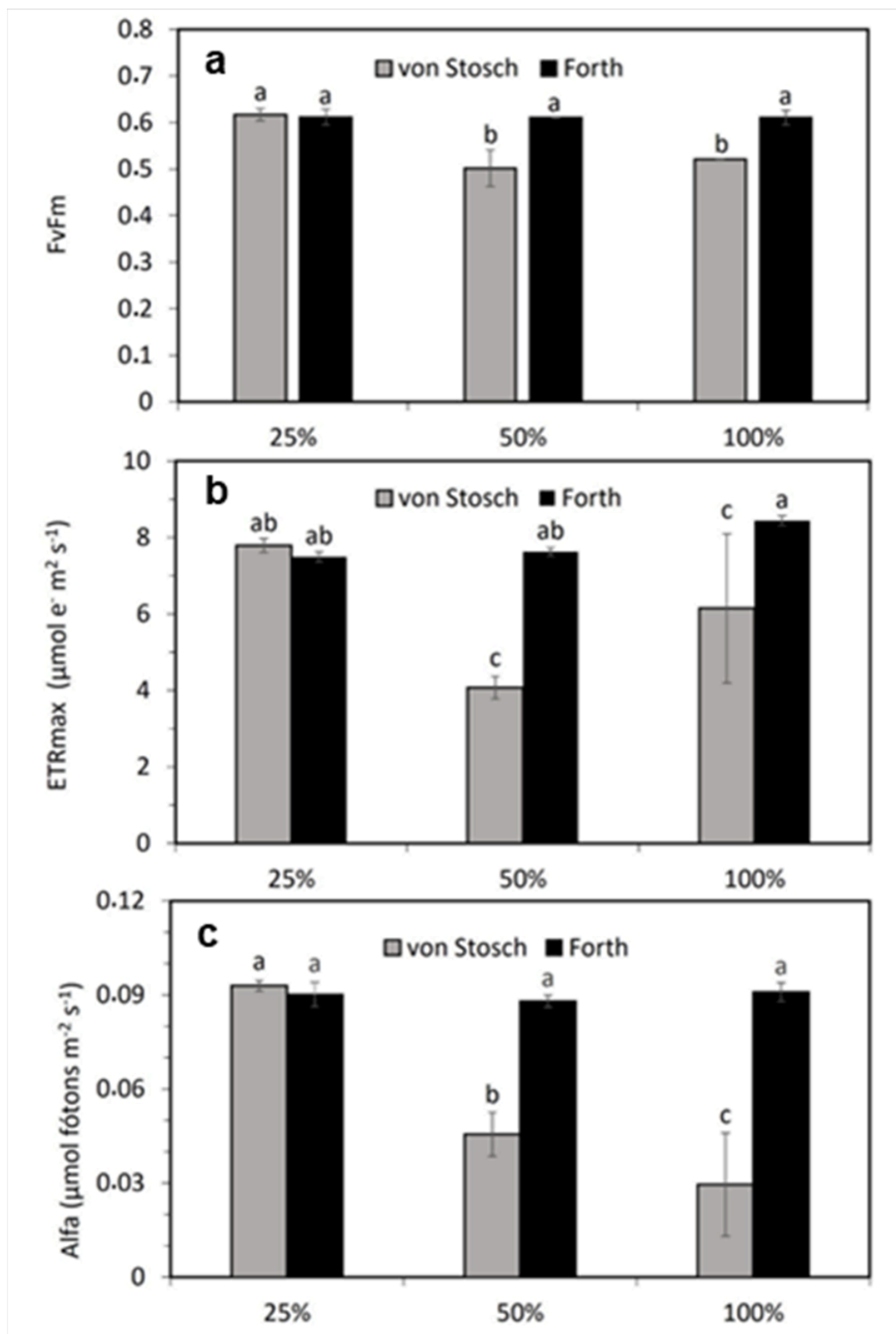


Figure 7

(a) Maximum quantum yield (Fv/Fm), (b) maximum ETR ($\mu\text{mol e}^- \text{m}^{-2} \text{s}^{-1}$), and (c) photosynthetic efficiency – alpha ($\mu\text{mol photons e}^- \text{m}^{-2} \text{s}^{-1}$) of *Gracilariopsis tenuifrons* in von Stosch (VS) and Forth (FT) treatments at concentrations of 25%, 50%, and 100% measured in week four after biomass removal. Values represent the mean and standard deviation ($n = 5$). Letters represent statistically differences ($p < 0.05$)

