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Original Article

Biomass, growth and nutritional composition of the seaweed *Gracilaria domingensis* (Kützing) Sonder ex Dickie (Rhodophyta) under different nitrogen and phosphorus availability

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ABSTRACT

Seaweeds have been used by several industrial sectors, such as the food, feed, pharmaceutical and biofuel industries. Thereby, techniques to increase seaweed production are needed due to the rising global demand for biomass. Thus, we investigated the effects of different weekly nutrient pulses [N and P at ratios of 10:1 (T1), 20:2 (T2), and 50:5 (T3)] on the biomass, relative growth rate (RGR) and biochemical composition of *Gracilaria domingensis*. A control without nutrient pulses was also established. The highest biomass values were recorded in T1. The RGR was more constant in T1 and T2 than in T3 throughout the cultivation. Significant decreases in RGR were observed in the control compared to the other treatments, and null RGR was recorded in T3. Regarding the seaweed biochemical composition, the lowest carbohydrate and lipid content and the highest ash content were recorded in T1. In our study, *G. domingensis* showed nutritional values similar or even superior to those reported for other seaweeds used as food. We concluded that T1 is the most suitable treatment to increase *G. domingensis* production. In addition to being the least expensive treatment, in T1, *G. domingensis* exhibited the highest biomass values, constant RGR, and nutritional composition suitable for human consumption.

Keywords: Biochemical composition, edible seaweed, macroalgae, nutrients, seaweed cultivation.

Introduction

Seaweeds are important natural resources from the oceans. Historically, they have been used as human food by several civilizations, mainly in Asian countries. In addition to being human food, seaweeds have been used in the production of animal feed and fertilizers, as well as for the extraction of phycocolloids (i.e., agar, carrageenan and alginate) and other bioactive compounds (Torres *et al.* 2019; Marinho-Soriano & Carneiro 2021). Several economic sectors have explored seaweed applications worldwide, such as food, cosmetics, pharmaceutical, textile, and biotechnology industries (Kılınç *et al.* 2013).

Seaweed growth is affected by several environmental factors, such as light, temperature, salinity, nutrients, and substrate (Smale *et al.* 2016). Nutrient availability is one of

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the main regulating factors of algal physiology in seaweed farming. The productivity, growth and biochemical content of farmed seaweeds are commonly affected by environmental concentrations of nutrients (Marinho-Soriano & Carneiro 2021). Usually, limiting concentrations of nitrogen (N) and phosphorus (P) can negatively affect seaweed development (Harrison & Hurd 2001). Thereby, under suitable environmental availability of nutrients, seaweeds can store large amounts of nitrogen and phosphorus in their tissues through various metabolic pathways. These stocks are commonly used to enhance the seaweed performance during periods with lower availability of these nutrients (Harrison & Hurd 2001, Marinho-Soriano *et al.* 2009). Nitrogen is crucial in the composition of proteins, nucleic acids and chlorophyll, while phosphorus is relevant for energy metabolism and the composition of nucleic acids and phospholipids (Hurd *et al.* 2014). Red seaweeds (i.e., Rhodophyta) have a high capacity to store nitrogen in phycobiliproteins, which are primarily accessory pigments. Under low environmental availability of nitrogen, these stocks can be used by red seaweeds to maintain their growth (Andría *et al.* 1999; Nagler *et al.* 2003; Fernandes *et al.* 2017).

A method used to enhance the productivity of farmed seaweeds has been nutrient pulses (Harrison & Hurd 2001). In the environment, seaweeds are already subjected to irregular nutrient releases, in other words, nutrient pulses into the system in natural or anthropogenic ways (Worm & Sommer 2000; Harrison & Hurd 2001). In aquaculture, nutrient pulses can be applied in a controlled and regular manner. This method consists of transferring seaweeds farmed under low nutrient concentrations to a nutrient-rich medium for a short time, and subsequently transferring them to the initial farming conditions. Thus, the farmed seaweeds uptake and store nutrients in their tissues, later using these stores for their growth (Harrison & Hurd 2001; Nagler *et al*. 2003; Fernandes *et al*. 2017). Nutrient pulses have also been used to manipulate the biochemical composition of farmed seaweeds. In seaweed farming, the frequency of pulses can increase the concentration of some compounds in the seaweeds, such as proteins, carbohydrates and vitamins (Nagler *et al*. 2003). Thereby, the success of seaweed farming will depend on knowledge about the ecophysiological properties of farmed species and how to manipulate physical and chemical factors to improve the growth and biochemical composition of seaweeds.

Recently, several studies have investigated the nutritional properties of seaweeds, as their use in human consumption is increasing. Seaweeds have a low lipid content and a high protein, fiber, vitamin and mineral content in their composition (Fleurence & Levine 2016; Kazir *et al.* 2019). They are considered a viable source of protein and some species even have levels similar to traditionally known sources, such as meat, egg, milk and soy (Bleakley & Hayes 2017). In addition to being rich in protein, seaweed is an excellent source of vitamins A, B (B_1, B_2, B_3, B_6, and B_{12}), C, D, and E (Škrovánková 2011). It has high levels of essential minerals for the human body, such as sodium, potassium, calcium, magnesium, iron and zinc (Tiwari & Troy 2015). Seaweeds also have a low caloric value, as their lipid content is commonly very low (1–5% of dry weight). Although they have a high carbohydrate content, especially fibers, with higher levels than those found in fruits and other vegetables (Fleurence & Levine 2016). For these reasons, the use of seaweed as a functional food is increasing, to ensure the daily intake of nutrients required by the human body (Tiwari & Troy 2015).

In this context, we investigated the effects of different nutrient pulses on the biomass, growth and biochemical composition of the red seaweed *Gracilaria domingensis* (Kützing) Sonder ex Dickie (Gracilariaceae) cultured under outdoor controlled conditions. The biochemical composition of the seaweed (proteins, lipids, carbohydrates, fibers and ashes) was determined to identify its nutritional value under different weekly pulses of nutrients. *Gracilaria domingensis* is a species widely distributed on the Brazilian coast and has great potential for human consumption (*in natura*) (Bellorin *et al*. 2002; Trigueiro *et al*. 2017).

Material and methods

Seaweed Collection

Gracilaria domingensis (Kützing) Sonder ex Dickie specimens were collected in Mãe Luiza beach (35°10'48.52"O; 5°47'57.59"S), Rio Grande do Norte state, Brazil. In this Brazilian region, there are two well-defined seasons: a rainy season from March to July and a dry season from August to February. Seaweeds were collected manually in August 2021 during low tide and subsequently transported to the laboratory of marine macroalgae located at the Department of Oceanography and Limnology of the Federal University of Rio Grande do Norte (DOL-UFRN). In the laboratory, epibionts were removed from branches and fertile plants (i.e., with prominent cystocarps) were discarded, as energy is directed toward reproduction at the expense of growth.

Experimental Cultivation

The outdoor cultivation was carried out at the Aquaculture Technology Center located at the DOL-UFRN. The experimental design consisted of twelve 10 L transparent glass aquaria containing 8 L of filtered seawater bubbled continuously with ambient air. In each aquarium, 24 g of vegetative branches were cultured (density of 3 g L^{-1}). Three treatments were established for the weekly pulses of nitrogen and phosphorus (N:P) at ratios of 10:1 (T1), 20:2 (T2), and 50:5 (T3). The target ratios were achieved by adding standard solutions of N-NH_4^+ and P-PO_4^{3-} to the seawater. The standard solutions were previously prepared using NH_4Cl and KH_2PO_4, respectively, as these compounds are widely used in similar approaches (*e.g.*, Joniyas *et al*. 2016; Han *et al*. 2023) and Cl^- e K^+ are abundant in natural seawater. Each treatment had three replicates ($n = 3$). A set of three aquaria was maintained as a control. Seaweeds cultured in the control were not subjected to weekly pulses of nitrogen and phosphorus.

Cultured seaweeds were weighed weekly on an analytical balance to determine their wet biomass. To standardize the wet weight, the excess water was removed by manual centrifugation (Salad Spinner, ~700 rpm, Motohashi 2020). Algal relative growth rates (RGRs = % d^{-1}) were calculated by applying the formula below, where Wi = initial wet weight, Wf = final wet weight, and $Tf - Ti$ = time interval (Marinho-Soriano *et al*. 2009):

$$RGR = \frac{ln\left(\frac{Wf}{Wi}\right)}{(Tf - Ti)} \times 100$$

After weighing, the seaweeds cultured in T1, T2 and T3 were subjected to nutrient pulses for two hours. Seaweeds were transferred from the aquaria to plastic containers (volume of 10 L) containing the nutrient-enriched seawater according to the respective treatment. The nutrient pulses were carried out under the same environmental conditions as outdoor cultivation. The enriched media were prepared as described above a few minutes before the pulses. During these procedures, all aquaria were cleaned and the seawater was renewed. After the pulses, seaweeds were washed with natural seawater and placed back into the aquaria according to the respective treatment. Throughout the outdoor cultivation, the photoperiod (around 12 h), the temperature of seawater (mean of 26 ± 1 ºC), salinity (40 PSU) and light intensity were natural. The cultivation lasted five weeks. At the end of cultivation, seaweeds were placed in transparent plastic bags and frozen at – 4 ºC. Afterward, they were dried in a laboratory drying oven at 60 ºC for seven hours. Finally, they were analyzed to determine their nutritional composition (proteins, lipids, carbohydrates, fibers and ashes) according to the AOAC methods (Latimer 2023). Before cultivation started, *G. domingensis* samples were also placed in transparent plastic bags and frozen at – 4 ºC for further biochemical analyses, following the same procedures mentioned above. These samples were used as an indicative of the initial biochemical composition of the seaweed.

Statistical analyses

The normality and homogeneity of variance of the data were tested by applying the Shapiro-Wilk and Levene tests, respectively. Repeated-measures of ANOVA were applied to determine significant differences for biomass and RGR in relation to the time (weeks) and cultivation conditions (control, T1, T2 and T3). Tukey's *post hoc* tests were applied when significant differences were found by the ANOVA. Significant differences between the initial and final biochemical composition of *G. domingensis* were determined by applying paired t-tests. The biochemical composition of *G. domingensis* among cultivation conditions over weeks was compared by applying repeated-measures of ANOVA followed by Tukey's *post hoc* tests when significant differences were found. The significance value adopted was 5% ($\alpha = 0.05$). All statistical analyses were performed using the R software.

Results

Biomass and RGR

The biomass and RGR results obtained from the control and treatments (T1, T2 and T3) during the *G. domingensis* cultivation are summarized in Table 1. The maximum biomass values registered in the cultivation conditions were 35.65 ± 1.58 g in T1, 32.52 ± 1.07 g in T2, 31.40 ± 0.98 g in T3, and 31.10 ± 0.90 g in the control. All maximum biomass values were observed in the last week of cultivation. Algal biomass increased significantly under all cultivation conditions with variation in weight gain over weeks (Fig. 1). *Gracilaria domingensis* exhibited the most constant biomass gain in T1, which resulted in the highest mean value of biomass in this treatment in the fourth week and at the end of cultivation (Table 1, Fig. 1). In other cultivation conditions the biomass gain varied among weeks, namely, the algal biomass decreased or did not vary in control, T2 and T3 among some weeks (Fig. 1).

In relation to algal RGR, branches cultured in T1 and T2 showed a more constant growth throughout the cultivation than those cultured in T3 and control (Fig. 2). The algal RGR did not differ between T1 and T2 over weeks. The maximum RGR values were recorded in T1 (1.26 ± 0.22 % d^{-1}) in the third week, and in T2 (1.39 ± 0.46 % d^{-1}) in the fifth week. The highest mean value of algal RGR was observed in T1 (Table 1). Algal RGR varied significantly in control and T3 over weeks ($F_{12,60} = 2.78$, $p = 0.007$). Null RGRs were registered for T3 in the third week of cultivation (Fig. 2). The algal RGRs decreased significantly in control throughout the cultivation (Fig. 2).

Nutritional Composition

The nutritional composition results of initial (after algal collection) and final (after cultivation) seaweed samples are summarized in Table 2. Carbohydrates increased significantly under all cultivation conditions (Table 2). The carbohydrate content in initial samples of *G. domingensis* was 29.31 ± 0.44 g 100 g^{-1} of dry weight (DW). The registered

Table 1. Biomass and relative growth rates (RGRs) of *Gracilaria domingensis* cultured for five weeks under weekly pulses of nitrogen and phosphorus in T1, T2 and T3. Values are minimum (Min), maximum (Max) and mean ± standard error (SE) based on three replicates (*n* = 3) from the control and treatments (T1, T2 and T3) during the cultivation. Branches were subjected to pulses of nitrogen and phosphorus at ratios of 10:1 (N:P), 20:2 and 50:5, in T1, T2 and T3, respectively. Branches were cultured without nutrient pulses in the control.

	Min – Max	Mean ± SE	ANOVA			
			Source of variation	df	F	*p**
Biomass (g)			Error: T	5		
Control	27.19 – 31.10	28.78 ± 0.90	Error: Within			
T1	27.20 – 35.65	30.36 ± 1.58	C	3	9.96	< 0.001
T2	26.50 – 32.52	28.30 ± 1.07	C × T	15	8.22	0.003
T3	26.53 – 31.40	28.03 ± 0.98	Residuals	48		
RGRs (% day^{-1})			Error: T	4		
Control	0.21 – 1.48	0.82 ± 0.19	Error: Within			
T1	1.01 – 1.26	1.16 ± 0.04	C	3	3.04	0.03
T2	0.93 – 1.39	1.15 ± 0.07	C × T	12	2.78	0.007
T3	0.00 – 1.19	0.82 ± 0.20	Residuals	40		

p values (*) indicate significant differences for biomass and RGRs among cultivation conditions over weeks by repeated measures of ANOVA. F refers to the calculated F and df to the degrees of freedom. T: Time (weeks), C: cultivation condition (control and treatments).

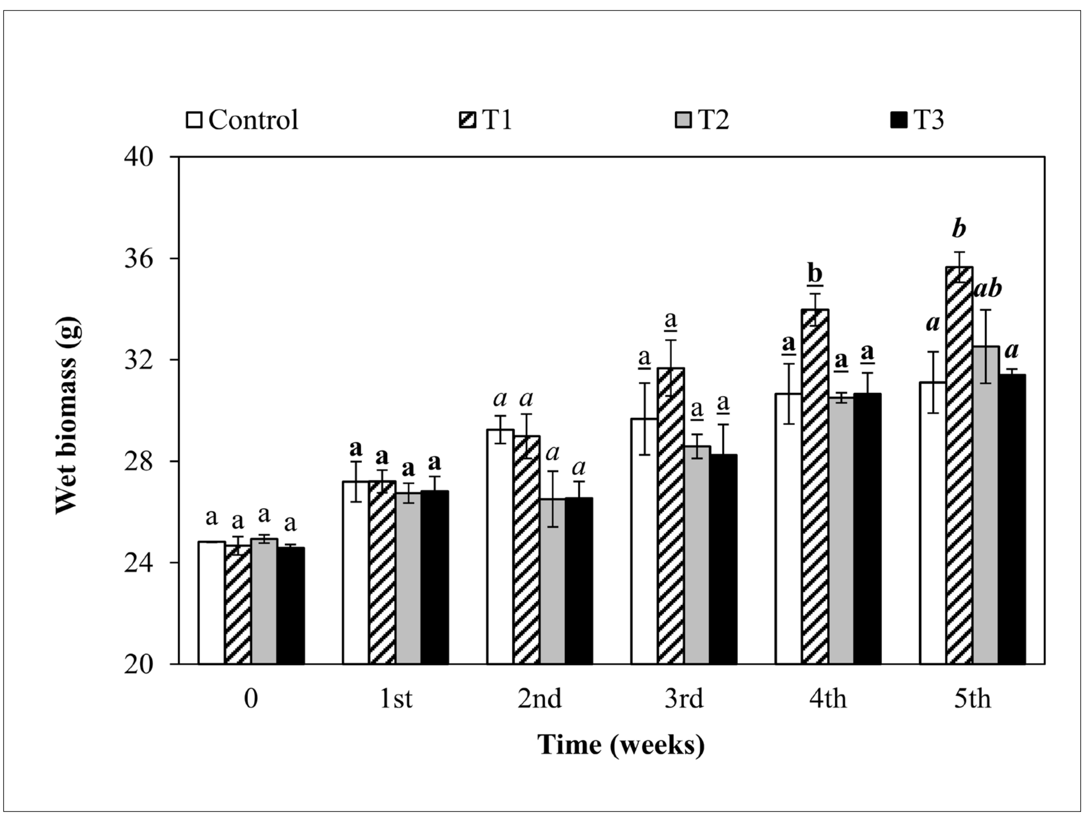

Figure 1. Biomass of *Gracilaria domingensis* cultured for five weeks under weekly pulses of nitrogen and phosphorus in T1, T2 and T3. Columns are means and bars are standard errors based on three replicates (*n* = 3) from the control and treatments (T1, T2 and T3) during the cultivation. Branches were subjected to pulses of nitrogen and phosphorus at ratios of 10:1 (N:P), 20:2 and 50:5, in T1, T2 and T3, respectively. Branches were cultured without nutrient pulses in the control. Different letters with the same formation indicate significant differences among cultivation conditions in the same week by repeated measures of ANOVA followed by Tukey's post hoc tests.

Table 2. Nutritional composition of *Gracilaria domingensis* collected in field (initial value) and after cultivation (final value) for five weeks under weekly pulses of nitrogen and phosphorus in T1, T2 and T3. Values are means ± standard errors based on three replicates (*n* = 3). Branches were cultured under weekly pulses of nitrogen and phosphorus at ratios of 10:1 (N:P), 20:2 and 50:5, in T1, T2 and T3, respectively. Branches were cultured without nutrient pulses in the control.

Biochemical compound (g 100 g^{-1} of dry weight)	Condition	Initial value	Final value	t value	p
Carbohydrates	Control	29.31 ± 0.25	44.86 ± 0.41[a]	-36.4	< 0.001*
	T1		35.88 ± 0.89[b]	-7.51	0.0173*
	T2		41.18 ± 0.77[c]	-11.9	0.0070*
	T3		40.46 ± 0.25[c]	-24.8	0.0016*
Fibers	Control	36.68 ± 0.37	41.68 ± 0.68[a]	-5.56	0.0115*
	T1		39.02 ± 0.64[a]	-2.99	0.0580
	T2		41.60 ± 0.79[a]	-6.52	0.0073*
	T3		41.57 ± 0.94[a]	-4.24	0.0240*
Proteins	Control	9.90 ± 0.03	9.99 ± 0.33[a]	-0.25	0.8238
	T1		10.58 ± 0.41[a]	-1.78	0.2174
	T2		10.73 ± 0.46[a]	-1.83	0.2073
	T3		10.97 ± 0.20[a]	-6.22	0.0249*
Lipids	Control	1.57 ± 0.06	0.66 ± 0.06[a]	9.47	0.0110*
	T1		0.59 ± 0.10[a]	7.13	0.0110*
	T2		1.78 ± 0.18[b]	-1.30	0.324
	T3		1.19 ± 0.06[c]	6.60	0.0222*
Ashes	Control	41.39 ± 0.28	26.66 ± 0.36[a]	25.8	0.0015*
	T1		35.12 ± 0.55[b]	15.3	0.0042*
	T2		28.48 ± 0.34[c]	21.8	0.0020*
	T3		29.56 ± 0.13[c]	47.7	< 0.001*

Asterisks (*) indicate significant differences between initial and final biochemical composition of *G. domingensis* by paired Student's t tests ($p < 0.05$). Different superscript letters indicate significant differences among cultivation conditions (control and treatments) for each biochemical compound by Tukey's *post hoc* tests ($p < 0.05$).

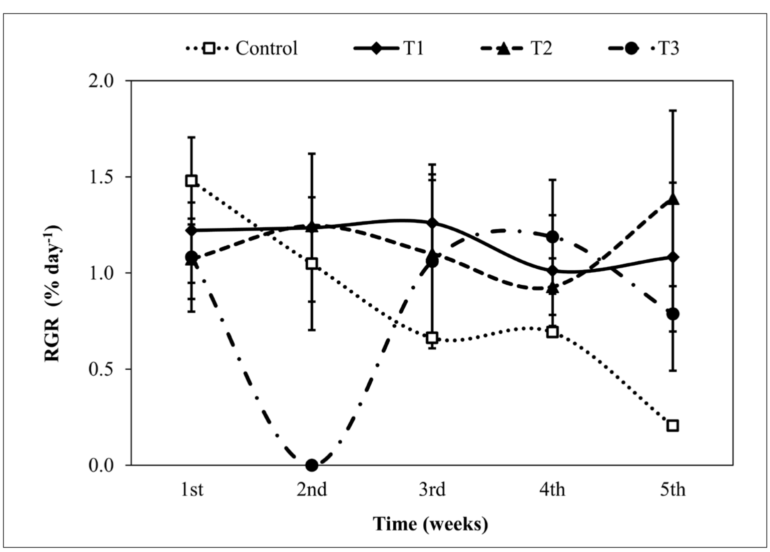

Figure 2. Relative growth rate (RGR) of *Gracilaria domingensis* cultured for five weeks under weekly pulses of nitrogen and phosphorus in T1, T2 and T3. Symbols are means and bars are standard errors based on three replicates (*n* = 3) from the control and treatments (T1, T2 and T3) during the cultivation. Branches were subjected to pulses of nitrogen and phosphorus at ratios of 10:1 (N:P), 20:2 and 50:5, in T1, T2 and T3, respectively. Branches were cultured without nutrient pulses in the control.

increase was highest in the control (44.86 ± 0.71 g 100 g^{-1} DW), followed by T2 (41.18 ± 1.34 g 100 g^{-1} DW), T3 (40.46 ± 0.43 g 100 g^{-1} DW) and T1 (35.88 ± 1.54 g 100 g^{-1} DW). The fiber content of *G. domingensis* also increased significantly under all cultivation conditions, except in T1 (Table 2). The protein content was relatively similar between initial (i.e., after algal collection) and final values (i.e., after cultivation), except in T3 (Table 2). In this treatment, the protein content increased significantly from 9.90 ± 0.06 g 100 g^{-1} DW to 10.97 ± 0.34 g 100 g^{-1} DW after cultivation (Table 2). Unlike carbohydrates, fibers and proteins, the lipid and ash contents of *G. domingensis* in general decreased significantly after cultivation (Table 2). The lowest lipid content was observed in T1 (0.59 ± 0.17 g 100 g^{-1} DW). The lowest ash content was observed in the control (26.66 ± 0.62 g 100 g^{-1} DW), whereas the highest was registered in T1 (35.12 ± 0.96 g 100 g^{-1} DW). T3 and T2 showed intermediate values, 29.56 ± 0.23 g 100 g^{-1} DW and 28.48 ± 0.59 g 100 g^{-1} DW, respectively.

A significant effect of weekly pulses of nitrogen and phosphorus on the nutritional composition of *G. domingensis* was found for carbohydrates, lipids and ashes in relation to the control (Table 2). The lowest carbohydrate and lipid contents were observed in T1. On the other hand, *G. domingensis* exhibited its highest ash content under this treatment.

Discussion

Biomass and RGR

Our study demonstrated that weekly pulses of nitrogen and phosphorus at the lowest concentration (i.e., N:P at ratios of 10:1 – T1) resulted in a positive effect on the seaweed biomass, especially from the third week, when seaweed biomass in T1 was higher than in the control (Fig. 1, Table 1). Regarding the growth, weekly pulses in T1 and T2 (N:P at ratios of 20:2) caused a more constant growth of *G. domingensis*. This evidence shows that weekly pulses as in T1 and T2 are more beneficial for seaweed growth than as in T3 (N:P at ratios of 50:5) and control (no nutrient pulses). These results are consistent with previous studies, which also demonstrated that the application of enriched culture media is necessary for seaweed cultivation (Hanisak 1990; Smale *et al.* 2016). Furthermore, these studies also demonstrated that the adequate nutrient concentration varies among species, thus being a species-specific ecophysiological response. Usandizaga *et al.* (2018) recorded significant positive effects of nutrient addition on the growth rate and productivity of *Gracilaria chilensis* C.J.Bird, McLachlan & E.C.Oliveira in experimental cultivation in southern Chile. Fernandes *et al.* (2017) also observed an increase in biomass and growth of *Gracilaria birdiae* E.M.Plastino & E.C.Oliveira under pulses from different nutrient sources in experimental cultivation in northeastern Brazil. Decreasing RGR was expected in the control, as in that treatment the seaweeds were cultured in natural seawater and were not subjected to weekly pulses of nitrogen and phosphorus.

Results found in our study have relevant technological applications, as the reduction in the use of fertilizers is directly reflected in the reduction of production costs in seaweed farming. Moreover, high nutrient concentrations do not necessarily result in increased seaweed growth. In addition to being more expensive, nutrient concentrations above the algal physiological tolerance limit can be harmful and significantly reduce the algal performance in cultivation (Usandizaga *et al.* 2018). Other negative effects of excess nutrients have also been discussed in the scientific literature, such as the development of opportunistic epiphytes, which compete for the resources of the medium (Worm & Sommer 2000; Harrison & Hurd 2001).

Nutritional Composition

When compared to the initial value (i.e., after algal collection), the concentrations (g 100 g^{-1} of dry weight) of carbohydrates and fibers increased significantly in *G. domingensis* under all cultivation conditions, especially in the control (Table 2). As in this condition the seaweed was not subjected to any nutrient pulse, it is possible to hypothesize that the low nutrient availability stimulated *G. domingensis* to accumulate energy reserves in the form of carbohydrates. Growth data confirm this observation since the RGR of *G. domingensis* decreased continuously in the control over cultivation (Fig. 2), and *G. domingensis* showed the highest carbohydrate content in this treatment at the end of cultivation (Table 2). The closely related seaweed *Gracilaria cervicornis* (Turner) J.Agardh also showed a similar relation between growth and carbohydrate content (Marinho-Soriano *et al.* 2006). Other authors also suggest that carbohydrate synthesis can be stimulated by decreasing nutrient availability and increasing light intensity and temperature (*e.g.* Rotem *et al.* 1986; Tabassum *et al.* 2016; Borburema *et al.* 2023). After the weekly pulses of nitrogen and phosphorus in the treatments, seaweeds were transferred to the oligotrophic conditions of cultivation. Consequently, they also increased their carbohydrate and fiber contents, although in lower values than in the control (Table 2). These results suggest an energy use for seaweed growth at the expense of carbohydrate accumulation. In our study, the carbohydrate content was lower in T1, where the seaweeds exhibited the highest biomass values and more constant growth.

Carbohydrates mainly play an energetic role in the human diet. In our study, the carbohydrate contents observed in *G. domingensis* were higher than those found in *Porphyra* spp. (“nori”) (i.e., 38.07 g 100 g^{-1} DW) and similar to those found in *Undaria pinnatifida* (Harvey) Suringar (“wakame”) (i.e., 41.03 g 100 g^{-1} DW), two seaweeds widely used as human food (Watanabe & Kawai 2018). In addition, seaweeds can also be an excellent source of fiber, since they have higher fiber contents than many traditional fiber-rich foods, such as beans (20.95 g 100 g^{-1} DW) (Sardinha *et al*. 2014), linseed (22.33 g 100 g^{-1} DW) and wheat germ (14.00 g 100 g^{-1} DW) (Dhingra *et al*. 2012). In our study, the fiber content found in *G. domingensis* was similar to that recorded in other edible seaweeds, such as *Laminaria digitata* (Hudson) J.V.Lamouroux ("kombu") (36.12%), *Undaria pinnatifida* (33.58%), *Chondrus crispus* Stackhouse (34.29%), *Neopyropia tenera* (Kjellman) L.-E.Yang & J.Brodie (as *Porphyra tenera* Kjellman) (33.78%) and *Hypnea pseudomusciformis* Nauer, Cassano & M.C.Oliveira (as *Hypnea musciformis* (Wulfen) J.V.Lamouroux) (37.92%) (Rupérez & Saura-Calixto 2001; Siddique *et al*. 2013). Fibers reduce the risk of cardiovascular diseases and diabetes, as they are not digested by the human body, they decrease the absorption of cholesterol and sugar (Anderson *et al*. 2009; Dhingra *et al*. 2012; Torres *et al*. 2019). Fibers are also known to benefit intestinal transit and maintain the microbiota (Dhingra *et al*. 2012).

In general, the protein content of *G. domingensis* was like that recorded by Torres *et al*. (2019) for this species. Nevertheless, in our study, *G. domingensis* showed a higher protein content than the closely related seaweed *G. birdiae* (Gressler *et al*. 2010). Compared to other edible seaweeds, *G. domingensis* showed a lower relative protein content than *Porphyra* spp. (24% – 44%), one of the most consumed seaweed worldwide (Sánchez-Machado *et al*. 2004; Smith *et al*. 2010; Cian *et al*. 2014; Paiva *et al*. 2014). However, the protein contents in *G. domingensis* were higher than those reported in *Laminaria digitata* (5% – 9%) (Kolb *et al*. 2004; Mæhre *et al*. 2014; Schiener *et al*. 2015), *Sargassum naozhouense* C.K.Tseng & Lu Baoren (11.20%) (Peng *et al*. 2013) and *Ulva lactuca* L. (9.56%) (as *Ulva fasciata* Delile) (Ismail 2017). The highest protein content of *G. domingensis* observed in T3, which differed significantly from the initial value (after algal collection), can be explained by the highest nitrogen availability in this treatment since nitrogen is used for protein biosynthesis.

Generally, seaweeds have a low lipid content. Although seaweeds have a lipid content of around 4%, they have relevant fatty acids, mainly omega-3 and omega-6 unsaturated fatty acids (Guaratini 2008). These fatty acids are very important for human health, as they decrease the risk of cardiovascular diseases and improve the immune system (da Costa *et al*. 2021). As expected, among the biochemical compounds analyzed in our study, lipids showed the lowest concentrations. The most significant decrease was observed in T1, where *G. domingensis* exhibited the highest biomass values at the end of cultivation. These results suggest that lipids were likely used to synthesize lipid-based structural biomolecules, such as the phospholipids that make up cell membranes since lipids are rarely found in the free state in algae (Khotimchenko 2005). Overall, the lipid content of *G. domingensis* was similar to that reported in the scientific literature for other Gracilariaceae species (Wielgosz-Collin *et al*. 2016). Similar results were also reported for *Condrus crispus* (1.7%), *Palmaria palmata* (L.) F.Weber and D.Mohr (1.6%) and *Porphyra* spp. (1.8%) from natural beds in Portugal (Campos *et al*. 2022), and *Devaleraea mollis* (Setchell & N.L.Gardner) G.W.Saunders, C.J.Jackson & Salomaki (as *Palmaria mollis*) (2.09%), cultured in the United States of America (Gadberry *et al*. 2018).

Seaweeds usually have a high ash content, as they uptake several inorganic compounds of seawater, which is rich in minerals (MacArtain *et al*. 2007; Chan & Matanjun 2017). Ashes are composed of minerals, such as sodium, magnesium, potassium, calcium, iron and zinc (Araújo *et al*. 2021). The ash content in *G. domingensis* after collection (i.e., initial value) was higher than after cultivation. This result can be explained because in the environment the minerals are continuously renewed, whereas in cultivation the seawater replacement was carried out weekly. In T1, *G. domingensis* showed a higher ash content than other *Gracilaria* species, such as *Gracilaria gracilis* (Stackhouse) Steentoft, L.M.Irvine & Farnham (24.8%) (Rodrigues *et al*. 2015) and *Gracilaria cornea* J.Agardh (29.06%) (Robledo & Freile-Pelegrín 1997). *Gracilaria domingensis* also exhibited a higher ash content than other traditionally consumed seaweeds, such as *Neopyropia tenera* (20.59 g 100 g^{-1} DW) (as *Porphyra tenera*), *Chondrus crispus* (21.08 g 100 g^{-1} DW) (Rupérez 2002), *Caulerpa lentillifera* J.Agardh (22.20%) (Nguyen *et al*. 2011) and *Ulva lactuca* (19.59%) (Yaich *et al*. 2011). Thereby, our results demonstrate that *G. domingensis* is an excellent source of minerals.

In conclusion, the cultivation of the red seaweed *G. domingensis* under weekly pulses of nitrogen and phosphorus resulted in a significant increase in biomass. Nevertheless, T1 (N:P at proportions of 10:1) showed the best biomass and growth results. In addition to improving the seaweed growth, weekly pulses of nitrogen and phosphorus enhanced the nutritional value of *G. domingensis*. The lowest carbohydrate and lipid contents were observed in T1, resulting in a lower caloric value, whereas the highest ash content was recorded under this treatment, indicating a high mineral content. Our study provides relevant information on the effects of controlled nutrient enrichment on the biomass, growth and nutritional quality of *G. domingensis*, and suggests that this seaweed has potential for aquaculture.

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Authors' Contributions

JNSB: Methodology, Formal analysis, Investigation, Writing – Original Draft, Visualization; HDSB: Visualization, Writing – Original Draft, Writing – Review & Editing; MAAC: Conceptualization, Methodology, Formal analysis, Investigation, Validation, Writing – Original Draft, Supervision; EM-S: Conceptualization, Methodology, Validation, Resources, Writing – Review & Editing, Supervision.

Conflict of interest

The authors declare they have no conflict of interests.

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