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Gracilarioid algae (Rhodophyta) cultured in eutrophic synthetic seawater: potential for growth and preliminary bioremediation assessment

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Abstract
Using synthetic seawater is an opportunity to turn marine aquaculture toward more independence from coastal areas and natural seawater. Although synthetic seawater can increase production costs, it is a reality for some marine ornamental aquaculture producers and aquarium operators. For example, the capacity of macroalgae to absorb dissolved inorganic nutrients is a desirable feature when using these plants in integrated multitrophic aquaculture systems. Thus, our aim was to evaluate the potential of culturing three distinct gracilarioid algae, *Gracilaria caudata*, *Gracilaria domingensis*, and *Gracilariopsis tenuifrons*, in eutrophic synthetic seawater collected from a running commercial clownfish aquaculture system. We also provide a preliminary assessment of their capacity to bioremediate inorganic nitrogen and phosphate under the same conditions. The experiment was carried out for 21 days, and all seaweeds grew and remained healthy. *Gp. tenuifrons* showed higher mean (± SD) growth rate (11.2 ± 1.1% day^{-1}), followed by *G. caudata* (7.8 ± 0.6% day^{-1}) and *G. domingensis* (4.6 ± 0.2% day^{-1}). *Gracilaria caudata* and *Gp. tenuifrons* showed higher absorption ($P < 0.05$) of ammonium and phosphate, with a mean reduction of 92.1% of NH_4^+-N and 3.4% of PO_4^--P. While all three species could be cultured in eutrophic synthetic seawater and take up ammonium and phosphate, *Gp. tenuifrons* showed the best growth and both *Gp. tenuifrons* and *G. caudata* showed better potential to bioremediate nutrients in these conditions.

Keywords Bioremediation · Seaweed culture · Rhodophyta · Ammonium removal · Phosphate removal · Recirculating aquaculture system

Introduction

The use of synthetic seawater can be an opportunity to promote marine land–based aquaculture. Current production is mostly based on recirculating aquaculture systems (RAS) because these closed systems with filtration allow an efficient use of water. It follows that RAS using synthetic seawater could reduce dependence on coastal areas and natural seawater, allowing marine aquaculture to move inland. Developing aquaculture in urban areas would have to confront the limitations of space, water, and energy. Nonetheless, making urban areas more productive could generate employment and take an important step towards the development of sustainable cities (Bunting and Little 2015).

One example of aquaculture in urban areas is the production of marine ornamental species. In contrast to aquaculture for human consumption, ornamental aquaculture requires

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little space, and the animals are sold per unit with a high value, tending to make this type of production economically viable (Wabnitz et al. 2003; Palmtag 2017). Marine ornamental aquaculture production systems often rely on synthetic seawater and use commercial salt for their marine aquariums (Rocha and Dinis 2017). Nowadays, a wide variety of commercial salt can supply this need and has been found suitable for rearing and breeding many sensitive marine species (Montalvo 2017). However, the use of RAS requires carrying out partial water changes constantly to avoid the excessive accumulation of nutrients, namely nitrate and phosphate. Thus, the volume of water is usually large during production cycles, putting water and salt among the highest production costs. Additionally, the discharge of eutrophic saltwater into public sewage and soil has negative ecological, social, and economic impacts (Tlusty 2002).

Integrated multitrophic aquaculture (IMTA) with macroalgae can make production more sustainable (Chopin et al. 2001). Macroalgae promote the cycling of dissolved inorganic matter (Chopin et al. 2001; Neori et al. 2004), reducing the need for water changes. Macroalgae also increase revenue as it is a product with economic value. Some studies have been conducted with seaweed nutrient uptake in RAS (Demetropoulos and Langdon 2004; Corey et al. 2014; He et al. 2014; Tremblay-Gratton et al 2018). Nonetheless, there are still knowledge gaps on the potential of cultivating some seaweed using synthetic seawater. Most studies with artificial seawater and macroalgae used different laboratory salt preparations (Kaladharan 2000; Mendes et al. 2012), with apparently few phycologists using ready-to-use commercial salts (Harrison and Bergs 2005). Thus, the recent availability of many reliable commercial salts for marine aquaria and its possibility of promoting land-based aquaculture opens the possibility of investigations into the cultivation of macroalgae in these conditions.

Red macroalgae of the order Gracilariales, such as those of the genera *Gracilaria* and *Gracilariopsis*, are mainly exploited for food and agar production (Oliveira and Plastino 1992; Oliveira et al. 2000; Marinho-Soriano 2017). Currently, around 10% of the world's production of seaweed is represented by red algae of the genus *Gracilaria* (FAO 2020) which, together with genus *Gracilariopsis*, is responsible for more than 90% of the agar value worldwide (Lim et al. 2017; Porse and Rudolph 2017). In Brazil, the exploitation of *Gracilaria* and *Gracilariopsis* species, mainly in the northeastern region, has had a serious impact on natural banks (Marinho-Soriano 2017). Among the main seaweeds of economic interest occurring on the Brazilian coast are *Gracilariopsis tenuifrons* (C.J. Bird & E.C. Oliveira) Fredericq & Hommersand, *Gracilaria caudata* J. Agardh, and *Gracilaria domingensis* (Kützing) Sonder ex Dickie. The first two are more used for agar, whereas *G. domingensis* is mainly used for food (Plastino et al. 1999; Oliveira et al. 2000; Simioni et al. 2019). Thus, the current exploitation of natural banks and the market diversity of these species make them interesting models for testing more sustainable methods of aquaculture production.

All phases of the life history of *Gp. tenuifrons* (Plastino et al. 1998; Yokoya 2000; Rossa et al. 2002; Torres et al. 2015), *G. caudata* (Oliveira and Plastino 1984; Miranda et al. 2012; Araujo et al. 2014; Faria and Plastino 2016; Faria et al. 2017; Marchi and Plastino 2020), and *G. domingensis* (Guimarães, et al. 1999; Plastino et al. 1999; Mendes et al. 2012; Ramlov et al. 2012; Castro and Yokoya 2019) can now be cultivated in the laboratory. To date, no large-scale commercial production of Gracilariales is found in Brazil (Marinho-Soriano 2017), only artisanal (Hayashi et al. 2014; Simioni et al. 2019). Species of *Gracilaria* have been studied in IMTA systems with fish and shrimp owing to their potential for absorbing nitrogenous inorganic compounds (Neori et al. 2004). Among the species that occur in Brazil, *G. birdiae*, *G. caudata*, *G. domingensis*, and, more recently, *Gp. tenuifrons* have been tested in an IMTA system, showing good growth and nutrient uptake capacity when cultured with shrimp in natural seawater (Marinho-Soriano et al. 2009, 2011; Trigueiro et al. 2017; Carneiro et al. 2021). Thus, future studies could focus on more diverse IMTA systems with seaweeds from the Brazilian coast. The possibility of using macroalgae for nutrient cycling could increase profitability, improve production, and reduce environmental impacts. Additionally, using synthetic seawater could promote new models of IMTA systems more independent from coastal areas and natural seawater. Thus, our aim herein was to evaluate the potential of culturing three distinct gracilarioid algae, *G. caudata*, *G. domingensis*, and *Gp. tenuifrons*, in eutrophic synthetic seawater from a commercial clownfish aquaculture. We further investigated, as a preliminary approach, their capacity to bioremediate inorganic nitrogen and phosphate, as an added benefit in these culture systems.

Material and methods

Source of algae and general culture conditions

All Gracilariales species used in this experiment were female gametophytes obtained from the Gracilariaceae Germplasm Bank of the University of São Paulo (Costa et al. 2012). Four individuals each of *Gracilaria caudata* (Ceará State, Brazil — 3.4°S 39.07°W; collected in 2012) and *Gracilaria domingensis* (Ceará State, Brazil — 3.4°S 39.07°W; collected in 1994), as well as three individuals of *Gracilariopsis tenuifrons* (Alagoas State, Brazil — 9°S 35.49°W; collected in 1993 and 1994) were selected. Tips of these unfertilized female gametophytes were grown separately in a unialgal and non-axenic culture to obtain the required

biomass for the study. Apical segments were maintained in Von Stosch–enriched seawater (Edwards, 1970) with small modifications (Ursi and Plastino 2001) and diluted to 50% with sterile natural seawater (salinity at 32). The algae were kept in a temperature-controlled room at 25 ± 1 °C with a photoperiod of 14:10 (light:dark). Photosynthetically active radiation (PAR) was set as 65 µmol photons m^{-2} s^{-1} using 40 W fluorescent tubes (Osram Daylight) measured by a quantameter (Li-COR model L1-185). Cultures received intermittent aeration (30 min h^{-1}) with atmospheric air by a radial blower, and the medium was totally renewed weekly. Voucher specimens can be found in the herbarium of the Bioscience Institute at the University of São Paulo (*G. caudata*, SPF-57842; *G. domingensis*, SPF-55809; *Gp. tenuifrons*, SPF-24001).

Source of eutrophic synthetic seawater

Eutrophic synthetic seawater was collected from a commercial clownfish grow-out system (Eco-reef, located in SP, Brazil) 6 days prior to the experiment. This commercial setup has approximately 3000 L of synthetic seawater with approximately 2500 fish in a recirculated system with biological and mechanical filtration. The company prepared this water using commercial salt for marine aquariums (Royal Nature® advanced pro formula; Israel) mixed with tap water purified by a reverse osmosis unit to achieve a salinity between 30 and 35 and pH around 8. We collected 80 L of water from this running system, which was filtered (10 µm mesh), sterilized in UV-C light, stored in plastic bottles (10 L each), and kept in the dark throughout the experimental period.

Experimental design

We set up four treatments — three algae species (*G. caudata*, *G. domingensis*, and *G. tenuifrons*) and a control (absence of seaweed) — with four replicates each during 21 days in a closed static system filled with eutrophic synthetic seawater. Thus, the experimental design was composed of a total of 16 experimental units completely randomized. Each experimental unit with algae corresponded to a 1-L Erlenmeyer flask stocked with four apical segments from different female gametophytes. For *Gp. tenuifrons*, we only had three different individuals (as described in "Source of algae and general culture conditions"); therefore, one individual was randomly chosen to be duplicated on every experimental unit. Consequently, this treatment also had four apical segments in each experimental unit. Because these algae species have different morphology, we standardized the size of apical segment within each species (10 mm for *G. domingensis*, 13 mm for *G. caudata*, and 50 mm for *Gp. tenuifrons*) so that a similar fresh mass would be shown among treatments. Thus, each group of four apical segments from the same species (experimental unit) had an initial mean fresh mass (± SD) of 18.2 ± 0.6 mg.

The experiment was carried out in a closed static system and under general culture conditions; however, no external nutrient or enriched medium was added at any time. The air flux was adjusted individually and was set to be just enough to provide uniform algae and water circulation without too much disturbance. The water in the experimental units was completely renewed weekly and replaced by the stored water (see "Source of eutrophic synthetic seawater"). Control treatment received the same conditions as others, but without algae. At the end of every week, we measured algal fresh mass, growth rate and productivity. To measure fresh mass, all segments from the same experimental unit were gently dried with a paper towel to remove excess water; the mass was evaluated with a scale (Mettler AE 200; 0.0001 g) and immediately returned to experimental conditions. Growth rates were estimated as $GR = [(Fm_t/Fm_0)^{1/t} - 1] \times 100$, where Fm_t is the final fresh mass, Fm_0 is the initial fresh mass, and t is time (Yong et al. 2013). Productivity was determined as total fresh mass produced per liter per day. The pH was measured with a multiparametric probe (HI98190 Hanna) on initial and final water every week. All flasks were covered with filter paper and cotton to minimize water evaporation and contamination from any other source.

Analysis of dissolved inorganic nutrients

We analyzed the dissolved inorganic nutrients on initial and final water every week. Water samples were filtered using an S-Pak filter membrane of 0.45 µm, preserved in polyethylene flasks and frozen (− 20 °C). Total phosphate phosphorus (PO_4^--P) concentrations were determined by the colorimetric method (Grasshoff et al. 1999) and spectrophotometrically read at 880 nm (± 0.01 µmol L^{-1}, Evolution 200 Thermo). Total ammonium nitrogen (NH_4^+-N) was analyzed by the method described by Tréguer and Le Corre (1975) and spectrophotometrically read at 630 nm (± 0.05 µM, Evolution 200 Thermo). Total nitrite nitrogen (NO_2^--N) and nitrate nitrogen (NO_3^--N) were measured automatically (AutoAnalyzer II Bran-Luebbe) using the colorimetric method (Tréguer and Le Corre 1975; Braga 1997a, b). Reduction of nitrate to nitrite was carried out through a cadmium-copper column (Wood et al. 1967). The precision of the method for nitrite was ± 0.01 µM, and for nitrate, it was ± 0.02 µM. We calculated the variation of nutrient (delta nutrient = Δnutrient) as the final value for nutrient concentration minus its initial value every week. It was considered

that algae absorbed some nutrient when the Δnutrient was negative and significantly higher than the control treatment.

Data analysis

Fresh mass, growth rates, productivity, and nutrient variation (Δnutrient) were analyzed by one-way ANOVA (Zar 2010). When significant differences were recorded, the Tukey HSD was used for post hoc comparisons. The level of significance considered was 95%.

Results

The nutrient concentrations from initial water remained stable throughout the experiment (mean values ± SD): 0.09 ± 0.01 mg L^{-1} of NH_4^+-N, 0.77 ± 0.01 mg L^{-1} of NO_2^--N, 54.1 ± 0.94 mg L^{-1} of NO_3^--N, and 7.86 ± 0.1 mg L^{-1} of PO_4^--P (Fig. 1). The pH from initial water also remained stable with a mean of 7.80 ± 0.08. The pH from final water showed no significant difference among treatments at the end of each week with a mean of 8.04 ± 0.02.

All seaweeds grew and remained healthy. *Gracilariopsis tenuifrons* attained higher mass (ANOVA: $F(2,8)=4.5$, $p=2\times10^{-5}$) at the end with a mean fresh mass (± SD) of 171.0 ± 29.0 mg, followed by *G. caudata* (87.4 ± 8.8 mg) and *G. domingensis* (46.6 ± 1.8 mg) (Fig. 2). The growth rate declined for all species (Fig. 1). Nonetheless, *Gp. tenuifrons* still showed higher growth rate during the whole experiment, ranging from 14.9 ± 1.6% day^{-1} in the first week (ANOVA: $F(2,9)=4.3$, $p=1\times10^{-5}$) to 8.2 ± 0.1% day^{-1} at the end (ANOVA: $F(2,8)=4.5$, $p=2\times10^{-7}$). Considering the total growth in 21 days, *Gp. tenuifrons* showed the highest mean of growth rate (11.2 ± 1.1% day^{-1}), followed by *G. caudata* (7.8 ± 0.6% day^{-1}) and *G. domingensis* (4.6 ± 0.2% day^{-1}) (ANOVA: $F(2,8)=4.5$, $p=4\times10^{-6}$). Productivity showed a constant increase for *Gp. tenuifrons*, reaching a mean (± SD) of 12.3 ± 2.3 mg L^{-1} day^{-1} at the end, which was higher than that of the others (ANOVA: $F(2,8)=4.5$, $p=3\times10^{-6}$) (Fig. 2). For *G. caudata* and *G. domingensis*, mean productivity showed no significant difference and remained stable at 3.0 ± 0.7 mg L^{-1} day^{-1} and 1.3 ± 0.2 mg L^{-1} day^{-1}, respectively (Fig. 2). *Gracilaria caudata* and *Gp. tenuifrons* showed higher variation of ammonium and phosphate, reducing a mean of 92.1 ± 5.4% of NH_4^+-N (mean absorption ± SD = 87.3 ± 7.5 µg) and 3.4% ± 0.9% of PO_4^--P (0.3 ± 0.1 mg) (Figs. 3 and 4). To a lesser degree, *G. domingensis* also showed a reduction of NH_4^+-N higher than that observed in control (Fig. 3). The variation of NO_2^--N and NO_3^--N showed some positive values, meaning that final nutrient concentration in these cases was higher than initial. Nonetheless, it is important to note that some variations in NO_2^--N and NO_3^--N represented less than 5% of the initial nutrient concentration. It was even possible to observe a reduction of NO_2^--N during the last week, with algae treatments showing higher variation than control (Fig. 3). As observed in the last 2 weeks, only treatment with *Gp. tenuifrons* showed a reduction of NO_3^--N with values higher than those of the other treatments (Fig. 3).

Discussion

Nutrient concentrations in the water collected from a commercial clownfish grow-out system and stored in the laboratory remained stable. This validates the method of filtering and stocking this water throughout the experiment. The commercial aquaculture from which we collected the water

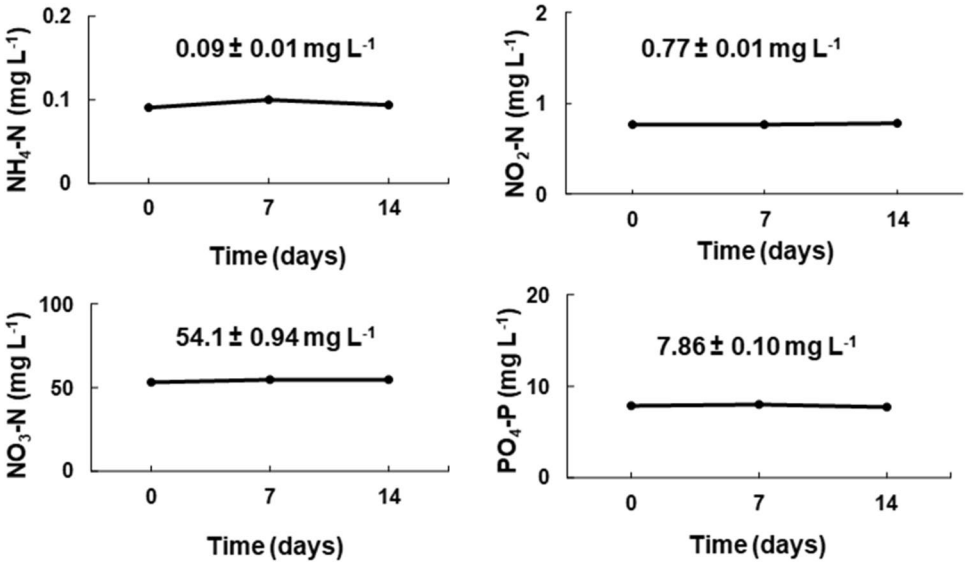

Fig. 1 Concentration of total ammonium nitrogen (NH_4^+-N), nitrite nitrogen (NO_2^--N), nitrate nitrogen (NO_3^--N), and phosphate phosphorus (PO_4^--P) in stored water used throughout the experiment for weekly renewal. The highlighted values are the nutrient concentration means ± standard deviation ($n=3$)

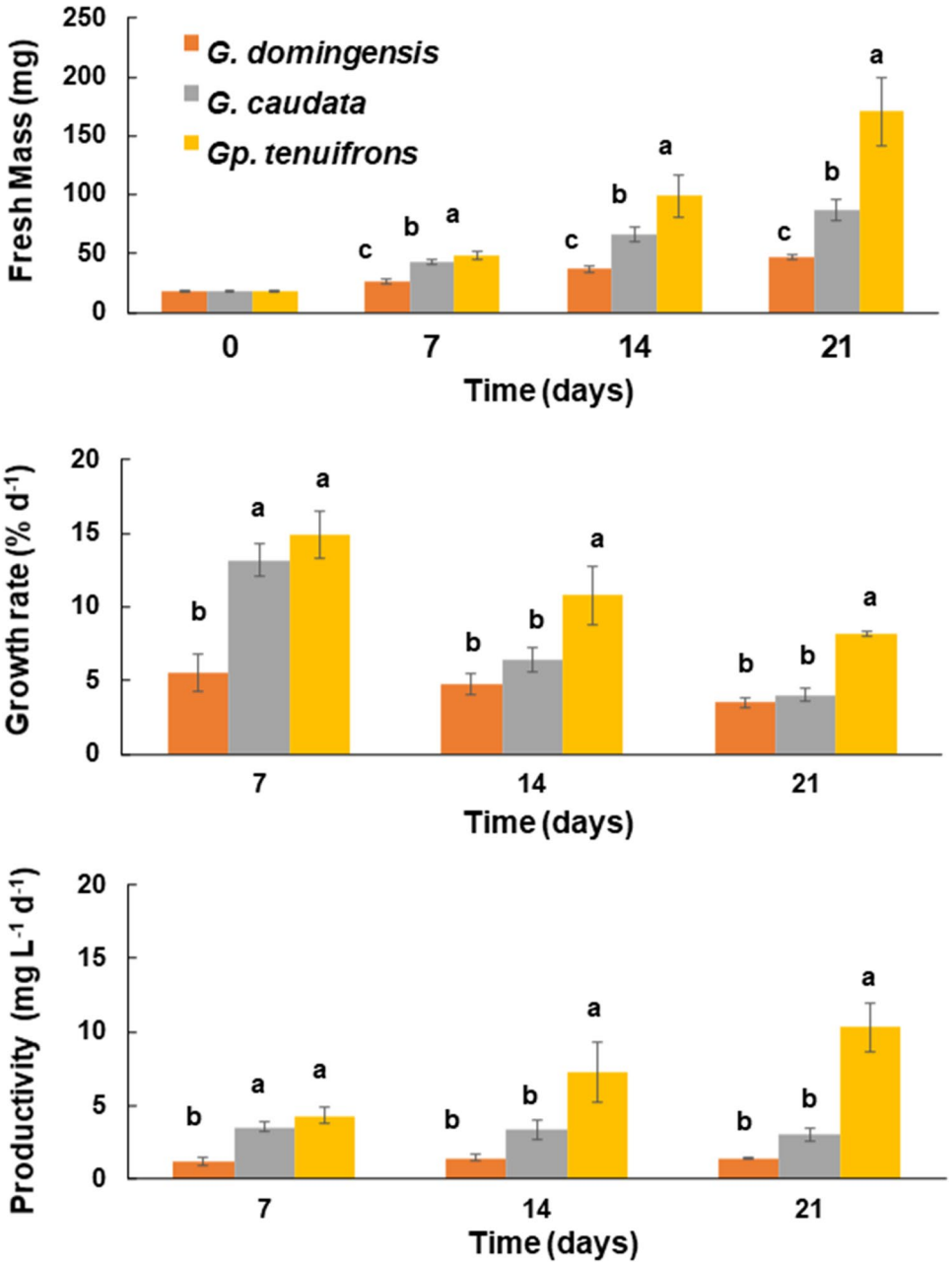

Fig. 2 Mean ± standard deviation ($n = 4$) of fresh mass, growth rate, and productivity of *Gracilaria caudata* (grey bars), *G. domingensis* (orange bars), and *Gracilariopsis tenuifrons* (yellow bars) cultivated for 21 days in a closed static system with eutrophic synthetic seawater. Letters indicate significant differences ($P < 0.05$) among treatments

uses a recirculating system with mechanical and biological filtration. Therefore, most accumulated nutrients were nitrate and phosphate. The nitrifying bacteria present in a well-established biofilter oxidize ammonia excreted by the fish to nitrite and then to nitrate, which accumulates (reviewed in Preena et al. 2021). This explains the lower values of ammonium and nitrite in the initial water when compared to nitrate. Phosphorous comes mostly from the leftover fish food and tends to accumulate in sediment and water, usually in the form of phosphate (David et al. 2017). Although these values of NH_4^+-N, NO_2^--N, NO_3^--N, and PO_4^--P are not toxic for fish, they are still much higher than the values found in natural seawater (e.g. mean nutrients concentration in oligotrophic seawater: 1.99 µmol L^{-1} of NH_4^+-N, 0.19 µmol L^{-1} of NO_2^--N, 0.13 µmol L^{-1} of NO_3^--N, and 0.5 µmol $L^{-1}$of PO_4^--P; Benitez-Nelson 2000; Braga et al. 2018). Thus, it was interesting to test this eutrophic synthetic water for culturing seaweed without adding any nutrient or enriched medium. Nonetheless, we recommend future studies to consider others macro and micronutrients, not considered herein, which may be important for seaweed health, especially at higher densities.

The fact that all macroalgae species in this work showed a constant increase in fresh mass indicates that the eutrophic synthetic seawater used here could efficiently maintain these seaweeds in the experimental conditions imposed. Indeed,

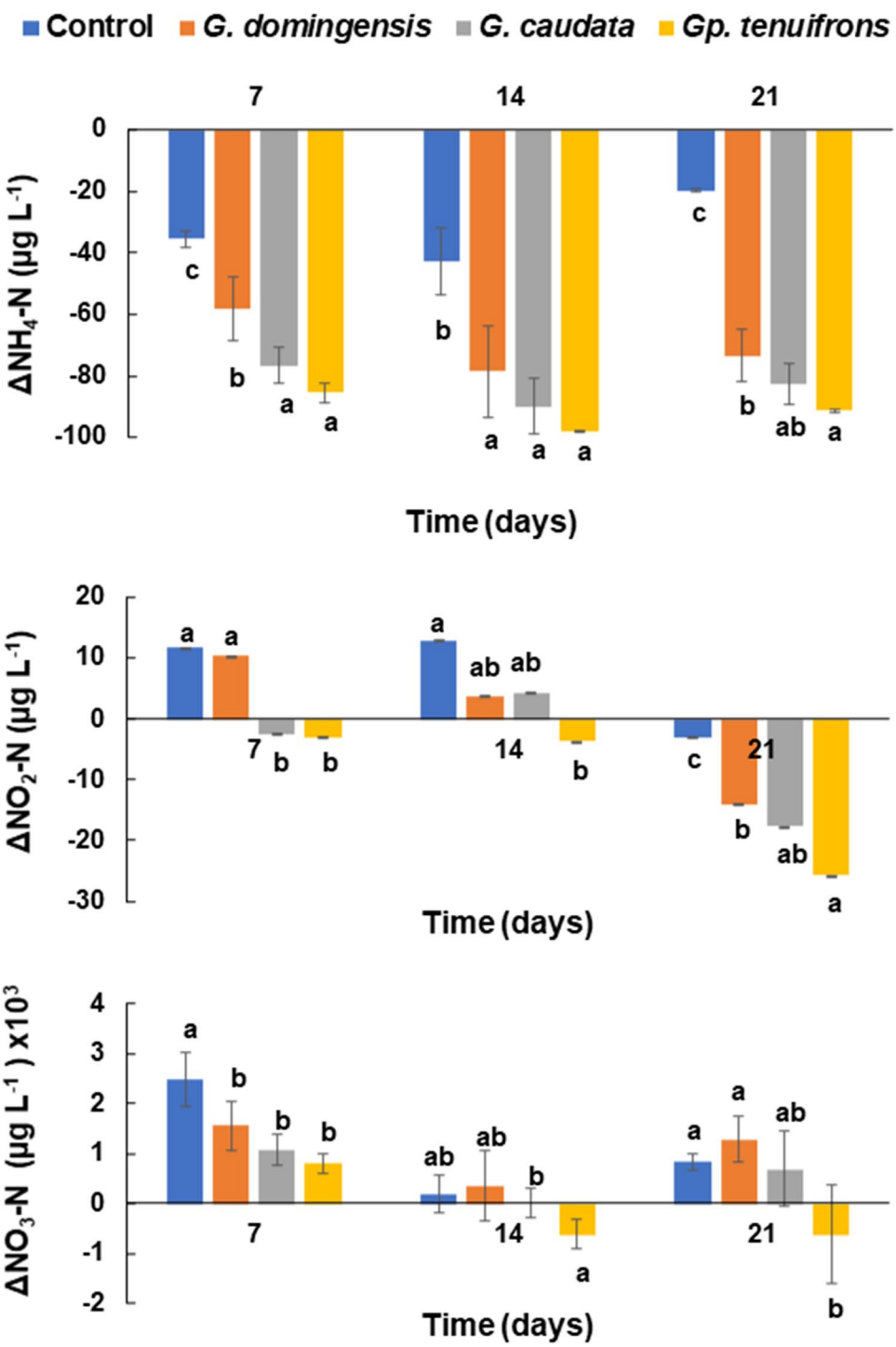

Fig. 3 Mean variation (Δnutrient ± standard deviation; $n = 4$) of total ammonium nitrogen (ΔNH_4^+-N), nitrite nitrogen (ΔNO_2^--N), and nitrate nitrogen (ΔNO_3^--N) on treatments with *Gracilaria caudata* (grey bars), *G. domingensis* (orange bars), and *Gracilariopsis tenuifrons* (yellow bars) cultivated for 21 days in a closed static system with eutrophic water. The control treatment (blue bars) represents the same conditions as others but without algae. Variation of a nutrient (Δnutrient) is the final value for the nutrient concentration minus its initial value on every week. Letters indicate significant differences ($P < 0.05$) among treatments

the mean growth rates (GR) for 21 days observed in this study (11.2% day^{-1} for *Gp. tenuifrons*; 7.8% day^{-1} for *G. caudata*; and 4.6% day^{-1} for *G. domingensis*) are similar to those published for the same species (also female gametophytes, but different populations) cultured in very similar conditions, but using Von Stosch–enriched natural seawater. These studies reported a mean GR of 12–13% day^{-1} for *Gp. tenuifrons* (Faria and Plastino, unpublished data), ~ 7% day^{-1} for *G. caudata* (Faria and Plastino, 2016), and 5.6% day^{-1} for *G. domingensis* (Guimarães and Plastino, 1999) also for 21 days. Although it is not possible to do a statistical comparison with the GR values found in these studies, the similarity among them shows that the experimental conditions used here did not limit algal growth and, thus, supports the hypothesis that eutrophic synthetic seawater using

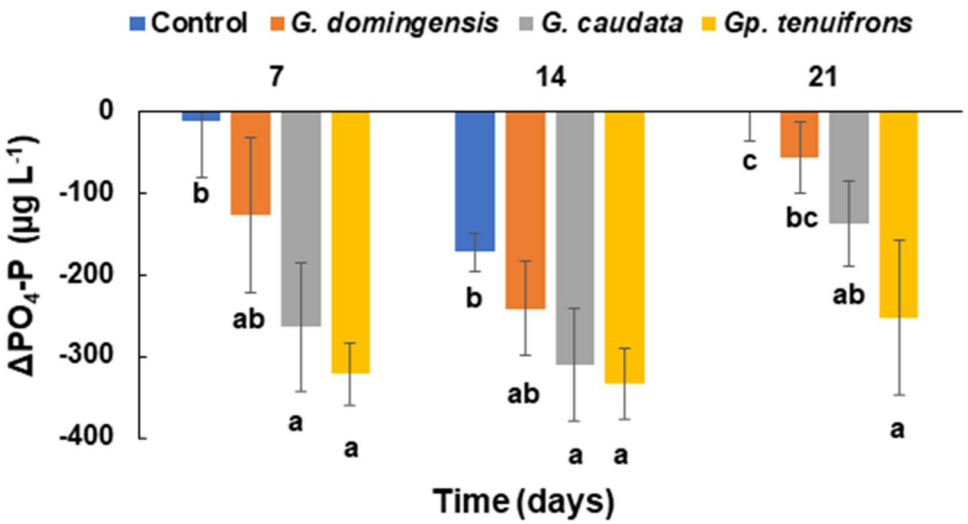

Fig. 4 Mean variation of phosphate phosphorus (ΔPO_4^--P ± standard deviation; $n = 4$) on treatments with *Gracilaria caudata* (grey bars), *G. domingensis* (orange bars), and *Gracilariopsis tenuifrons* (yellow bars) cultivated for 21 days in a closed static system with eutrophic water. The control treatment (blue bars) represents the same conditions as others but without algae. Variation of phosphate phosphorus is the final value for this nutrient concentration minus its initial value on every week. Letters indicate significant differences ($P < 0.05$) among treatments

commercial salt for aquariums might be suitable for the cultivation of seaweeds.

Among the three species tested, *Gp. tenuifrons* showed the best results in terms of increases in fresh mass, growth rates, and productivity. Nonetheless, the comparison should be interpreted cautiously. To explain, even with initial fresh mass being standardized among algae treatments, the difference in morphology, or even physiology, can naturally favor the growth of a particular species. This corroborates studies cited above, in which *Gp. tenuifrons* also showed higher growth when compared to *G. caudata* and *G. domingensis*, even when using natural seawater with enriched medium. Although these three species are branched and have apical growth, *Gp. tenuifrons* and *G. caudata* have cylindrical and thin thallus, whereas *G. domingensis* has a flat and thicker thallus. Moreover, the latter has a lower surface/volume. These characteristics may explain the lowest growth rates observed in *G. domingensis*. Moreover, *Gp. tenuifrons* is thinner than *G. caudata*, showing the highest surface/volume, which could favor its best performance. Placing this aside, the present study aimed to evaluate if these three different species could thrive in eutrophic synthetic seawater, and based in our results, we can suggest the use of this commercial salt for gracilarioid algae in general.

The reduction in nutrient concentration was more clearly observed for NH_4^+-N and PO_4^--P. Some ammonium and phosphate reduction was also observed in the control treatment (without seaweeds). Thus, we cannot rule out the possibility of nitrifying bacteria oxidizing ammonium to nitrate; in fact, it is expected that both processes, absorption by the algae and nitrification, will occur simultaneously. The reduction of PO_4^--P observed in control, most evident in the second week, could be explained by possible adsorption of this nutrient by some used material, perhaps the porosity in the glass flasks (Dalas and Koutsoukos 1990), sample vials, or aeration tubes. Nonetheless, except for the PO_4^--P in the *G. domingensis* treatment, all algae treatments showed higher reduction of NH_4^+-N and PO_4^--P when compared to control. Thus, even with nitrification activity and phosphorous adsorption, it can be concluded that *Gp. tenuifrons* and *G. caudata* absorbed most of both nutrients and that *G. domingensis* absorbed at least some ammonium. Although both *Gp. tenuifrons* and *G. caudata* showed the highest absorption when compared to *G. domingensis*, this difference is biased owing to the difference in algal GR. Because *G. domingensis* showed the lowest GR, we should not conclude that it has inferior bioremediation capacity. It is expected, however, that algae with higher surface/volume ratio showed faster uptake of nutrient (Kain and Norton 1990). *Gracilaria caudata* had a lower GR than *Gp. tenuifrons*, but *Gp. tenuifrons* and *G. caudata* still showed no significant difference in nutrient absorption. Therefore, it could be assumed that *G. caudata* may have a better bioremediation capacity than *Gp. tenuifrons* based on its mass, but this still needs further investigation.

The variation of NO_2^--N and NO_3^--N concentrations showed no conclusive results. Nitrite is unstable and, depending on the oxidation–reduction potential, could either turn to ammonium in the absence of oxygen or into nitrate in the presence of oxygen (Payne 1973; Preena et al. 2021). Because all experimental units were aerated and both water and algae were in constant movement, it is safe to assume that the predominant pathway would be the oxidation of ammonia to nitrite and then to nitrate. Indeed, except for *Gp. tenuifrons*, most nitrate showed an increase in all treatments. The reduction of NO_3^--N observed in the last 2 weeks for *Gp. tenuifrons* may indicate the absorption of this nutrient by the seaweed. This does not mean that the other seaweeds were not absorbing nitrate, but that it is not possible to distinguish it from nitrification in this experiment, possibly due to the lower algae density used.

Some red seaweeds have preference for ammonium over nitrate due to the absence of energy cost (Kain and Norton 1990; Ribeiro et al. 2017). Apparently, this seems to be the case of the three tested species. The mean absorption close to 90% of the NH_4^+-N available shows that the full absorption capacity of these algae, namely *Gp. tenuifrons* and *G. caudata*, might not have been achieved in this experiment. Indeed, the possible depletion of ammonium in the last 2 weeks by *Gp. tenuifrons* might explain the higher reduction of NO_3^--N in the same period. Thus, future experiments should consider evaluating higher initial concentrations of ammonium and/or higher density of algae to better understand the kinetics of nutrient uptake.

This study was only a preliminary approach to determine if gracilarioid algae could thrive in eutrophic synthetic seawater made with commercial salt and, if so, further determine if the algae have sufficient potential to absorb dissolved inorganic nutrients under these conditions. Our results suggest that these three species can be cultured in eutrophic synthetic seawater and absorb ammonium and phosphate. Apparently, *Gp. tenuifrons* showed better indicators of growth, while both *Gp. tenuifrons* and *G. caudata* showed better potential to bioremediate nutrients under study conditions. Thus, our results provide solid guidelines for future studies of integrated multitrophic aquaculture with synthetic seawater to investigate higher density of algae. The possibility of growing macroalgae in eutrophic synthetic seawater using commercial salt for marine aquariums is still incipient and has many applications. For example, macroalgae could be integrated with marine fish in an aquaculture system that relies on synthetic seawater for production, such as marine ornamental aquaculture in urban areas. Seaweeds would provide nutrient cycling, reducing costs associated with water exchange. Another application of synthetic seawater with commercial salt would be the promotion of better control over experimental conditions with dissolved kinetics of nutrients. It is also an alternative for those laboratories without access to good quality natural seawater or unable to sterilize seawater.

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

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