

Article

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Effects of nitrate and phosphate availabilities on growth, photosynthesis and pigment and protein contents in colour strains of *Hypnea musciformis* (Wulfen in Jacqu.) J.V. Lamour. (Gigartinales, Rhodophyta)

Aline P. Martins,¹ Orlando Necchi Junior,² Pio Colepicolo,³ Nair S. Yokoya^{*1}

¹Núcleo de Pesquisas em Ficologia, Instituto de Botânica, Secretaria de Estado do Meio Ambiente, Brazil,

²Departamento de Zoologia e Botânica, Instituto de Biociências, Letras e Ciências Exatas, Universidade Estadual Paulista Júlio de Mesquita Filho, Brazil,

³Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo, Brazil.

Abstract: In Brazil, *Hypnea musciformis* is the main raw material for carrageenan production and the knowledge of nitrogen and phosphorus metabolism in algae is critical for the success of cultivation because these elements can limit seaweed productivity. Thus, the objective of this study was to evaluate the effects of nitrate (zero to 100 μM) and nitrate plus phosphate (zero to 25 μM) availabilities on the growth, the contents of photosynthetic pigments (phycobiliproteins and chlorophyll a) and proteins, and the photosynthesis and respiration of the brown (BR) and light green (LG) strains of *H. musciformis*. The results revealed metabolic differences between the colour strains of *H. musciformis* for nitrogen metabolism: upon nitrate addition, the LG strain stored nitrogen mainly as proteins, while the BR strain stored it as proteins and pigments. Moreover, the respiration of the LG strain and the photosynthesis of the BR strain increased with nitrate concentrations, indicating that the BR strain fixed more photosynthetic carbon than the LG strain.

Introduction

The genus *Hypnea* (Gigartinales, Rhodophyta) includes about fifty species that are distributed in warm water areas (Masuda et al., 1997), of which six species are known in Brazil (Schenkman, 1986; Nunes, 2005). *Hypnea musciformis* (Wulfen in Jacqu.) J. V. Lamour. is a species with wide geographical distribution along the Brazilian coast and is the main raw material for carrageenan production (Oliveira, 1998). Carrageenan is a sulfated polysaccharide widely used in the food and pharmaceutical industries; it also has antiviral activity, inhibiting, for example, HSV and HIV (Neushul, 1990).

The concern with the impoverishment of natural beds of *H. musciformis* led to several investigations, which found that there are large variations in biomass and carrageenan yields during the year related to seasonality (Schenkman, 1989; Faccini & Berchez, 2000; Ramos et al., 2006). Since the carrageenan production from natural populations is very variable, studies were undertaken

to determine the best conditions for *H. musciformis* cultivation in the laboratory, and in the ocean (Faccini & Berchez, 2000; Reis et al., 2003; Schenkman, 1989). However, few studies evaluated the nutrient requirements of this species.

Nitrogen is considered to be the primary limiting nutrient for algal growth in marine ecosystems. Thus, the success of seaweed cultivation requires a knowledge of the nitrogen requirements of the algae (Hanisak, 1990). In the absence of this nutrient, many algae present the following changes: hair formation (DeBoer & Whoriskey, 1983; O'Connor & West, 1991); a decrease in growth (Hwang et al., 1987; Collén et al., 2004); a decrease in the contents of soluble proteins and phycobiliproteins (Collén et al., 2004); a decrease in the nitrate, nitrite and aminoacid contents of the thallus (Hwang et al., 1987); a decrease in photosynthesis and in the activities of enzymes involved in carbon metabolism (Collén et al., 2004); and a decrease in nitrate reductase activity, involved in nitrogen assimilation (Wheeler & Weidner,

1983).

Phosphorus is another important nutrient, since it participates in the formation of biomolecules such as nucleic acids, proteins and phospholipids. However, the most important role is in energy transfer mediated by ATP and other high energy compounds present in photosynthesis and respiration (Lobban & Harrison, 1994). Phosphorus enrichment can stimulate the growth and the photosynthetic rates of some algae (Lapointe, 1986), as well as increase carrageenan production (Chopin & Wagey, 1999). However, enrichment with high phosphate concentrations reduced the seedling survival of *Fucus vesiculosus* L. by 50% (Bergstrom et al., 2003).

Considering the importance of a knowledge of the nutrient requirements of seaweeds, the objective of this study was to evaluate the effects of nitrate and phosphate availabilities on the growth rate, on the contents of photosynthetic pigments and proteins, and on photosynthesis and respiration in a phycoerythrin deficient strain (light green strain, LG) and a wild strain (brown strain, BR) of *Hypnea musciformis*.

Materials and Methods

Algal material and unialgal cultures

The study was conducted with a wild strain (brown, BR) and a phycoerythrin deficient strain (light green, LG) of *Hypnea musciformis* (Wulfen in Jacqu.) J. V. Lamour. The LG strain is a phycoerythrin-deficient mutant derived from a green branch that appeared as a spontaneous mutation in a brown tetrasporophyte collected at Ponta da Baleia, Espírito Santo, Brazil. Both isolates were obtained by vegetative propagation in the laboratory. Voucher specimens have been deposited in the SP herbarium under the numbers SP365645 (LG) and SP365646 (BR).

Culture medium was composed of sterilized seawater (salinity 30 psu) enriched with von Stosch's solution at half strength (VSES/2) following Edwards (1970), with vitamin concentrations reduced to 50%. Medium renewal was carried out every week. Cultures were incubated at 24 °C under irradiances of 80.0-90.0 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, provided by cool-white fluorescent lamps with a 14:10 h light:dark cycle, without aeration.

Experiments with different nitrate and phosphate concentrations

These experiments were conducted with starved specimens, which were grown in sterilized seawater without nutrient addition for two weeks prior to the experiments.

Treatments consisted of sterilized seawater

enriched with different nitrate concentrations (NaNO_3 , zero, 20, 40, 60, 80, and 100 μM) or a combination of nitrate plus phosphate ($\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$) at a constant N:P ratio of 4:1 (N:P = 0:0, 20:5, 40:10, 60:15, 80:20, and 100:25 μM). The following variables were analysed: growth rate, protein and pigment content and photosynthesis and respiration. At the end of the experiment (28 days), samples were stored at -20 °C for subsequent analysis.

Growth rates

Growth rates (GR) were calculated as $[\ln(\text{Bf} \cdot \text{Bo}^{-1}) \cdot t^{-1}]$, where Bo is the initial fresh biomass, Bf is the fresh biomass after t days, and t corresponds to the experimental period (Yokoya et al., 2003).

Pigment and total soluble protein contents

Extractions of phycobiliproteins were carried out at 4 °C, according to Kursar et al. (1983) with modifications (Plastino & Guimarães, 2001). The algal mass (75 mg fresh mass for each replicate) was ground to a powder with liquid nitrogen and mixed with 50 mM phosphate buffer (pH 5.5). The homogenates were centrifuged at 36,000 g for 25 min in order to separate the phycobiliproteins present in the supernatants. Chlorophyll *a* was extracted after dissolving the pellet in 90% acetone and centrifuging at 12,000 g for 15 min. Pigments were quantified by spectrophotometry, concentrations being calculated according to Kursar et al. (1983) for phycobiliproteins (phycoerythrin - PE, phycocyanin - PC and allophycocyanin - APC) and to Jeffrey & Humphrey (1975) for chlorophyll *a* (Cl *a*).

For total soluble protein analyses, the algal biomass (75 mg fresh mass) was ground with liquid nitrogen, and extractions were carried out at 4 °C using 0.2 M phosphate buffer (pH 8) containing 5 mM EDTA and 1 mM DTT. Buffer was added in the proportion of 10 mL g^{-1} fresh biomass and the homogenates were centrifuged at 12,000 g for 20 min. Total soluble protein contents were determined according to Bradford (1976), using a Bio-Rad protein assay kit and BSA as standard.

Determination of photosynthesis and respiration

Photosynthesis was estimated by the oxygen evolution technique. Photosynthesis and dark respiration rates were determined from the changes in oxygen concentrations using the light and dark bottle technique (Littler & Arnold, 1985; Thomas, 1988). Initial and final oxygen concentrations were measured with an oxygen meter (Model 5000; Yellow Springs Instruments, USA) equipped with a self-stirring probe. Incubation periods were 30 min for each treatment and 1 h for dark respiration.

Statistical analyses

All treatments were performed in triplicate. Data were submitted to analysis of variance (ANOVA) of one factor, followed by Tukey's multiple comparison test, and ANOVA of two factors, followed by Student-Newman-Keuls' comparison test, considering the confidence level of 95%. Pearson's correlation was conducted for the variables analyzed in the experiments. Statistical tests were performed using SigmaStat software (version 1.0).

Results

Effects of nitrate availability

The growth rates (GR) of the LG strain were higher in the treatments with 20, 60, 80 and 100 μM nitrate and of the BR strain in treatments with 20, 80 and 100 μM nitrate (Figure 1). The BR strain showed a statistically significant higher GR than the LG strain in the treatment with 20 μM nitrate.

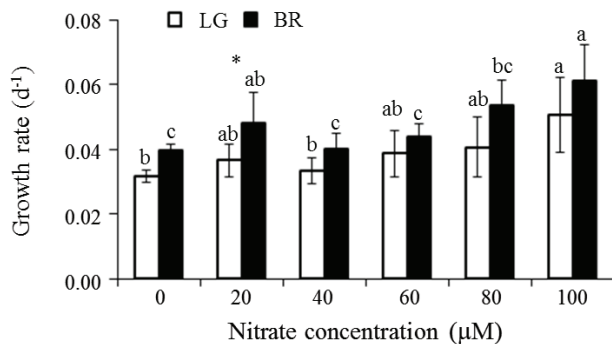


Figure 1. Growth rates of the light green (LG) and brown (BR) strains of *Hypnea musciformis* cultured for 28 days in sterile seawater enriched with different nitrate concentrations. Each data point is the mean of three replicates and bars are the standard deviation. Treatments with distinct letters indicate significant differences according to the one-way ANOVA, and Tukey's multiple comparison test ($p < 0.05$). *Indicates significant differences between strains, according to the comparison test of Student-Newman-Keuls.

The LG strain presented phycoerythrin (PE) only in the control treatment; upon addition of nitrate to the medium, the content of this phycobiliprotein was below the limit of detection. The phycocyanin (PC) and allophycocyanin (APC) contents were higher in treatments with 20, 40, 60 and 80 μM nitrate, except for APC in the treatment with 80 μM nitrate. The BR strain showed lower and higher contents of phycobiliproteins in the control and the treatments with nitrate (20 to 100 μM), respectively (Figure 2). There were no differences

in the Cl a contents for the different treatments.

A comparison of the two strains indicates that the LG strain had a higher content of APC than the BR strain in the control and in the treatment with 20 μM nitrate, of PC in the control and in the treatment with 20 and 60 μM nitrate and of Cl a in the treatment with 20 μM nitrate (Figure 2). The BR strain had a higher content of PE than the LG strain in all treatments.

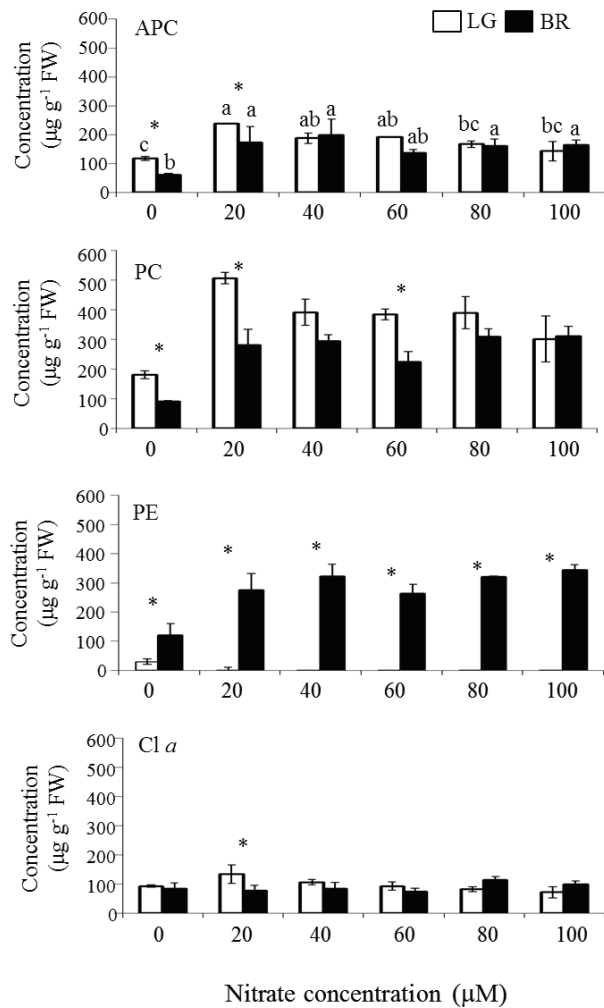


Figure 2. Concentrations ($\mu\text{g g}^{-1}$ fresh weight) of light-harvesting pigments in the light green (LG) and brown (BR) strains of *Hypnea musciformis* cultured for 28 days in sterile seawater enriched with different concentrations of nitrate. Each data point is the mean of three replicates and bars are the standard deviation. Treatments with distinct letters indicate significant differences according to the one-way ANOVA, and Tukey's multiple comparison test ($p < 0.05$). *Indicates significant differences between strains, according to the comparison test of Student-Newman-Keuls.

Both strains showed the lowest content of total soluble proteins in the control treatment and the highest content from 20 to 100 μM nitrate (Figure 3). Comparing the two strains, the BR strain has a higher

content of soluble protein than the LG strain in the treatment with 80 μM nitrate.

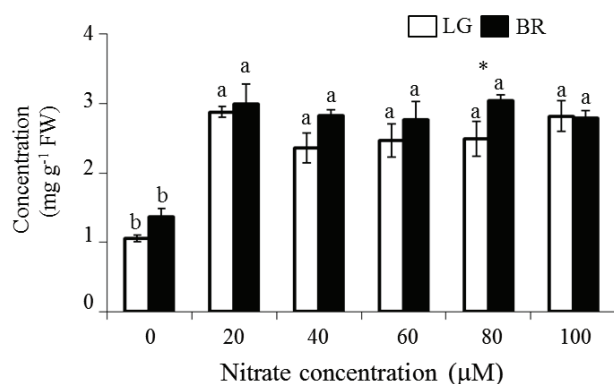


Figure 3. Concentrations (mg g^{-1} fresh weight) of soluble proteins in the light green (LG) and brown (BR) strains of *Hypnea musciformis* cultured for 28 days in sterile seawater enriched with different concentrations of nitrate. Each data point is the mean of three replicates and bars are the standard deviation. Treatments with distinct letters indicate significant differences according to the one-way ANOVA, and Tukey's multiple comparison test ($p < 0.05$). *Indicates significant differences between strains, according to the comparison test of Student-Newman-Keuls.

The optimum nitrate concentration net photosynthesis (P) by the LG strain was 20 μM nitrate; at higher concentrations, P began to decrease and reached the lowest value at 100 μM nitrate. For the BR strain, P was lower in the control and greater at 20, 60 and 100 μM nitrate (Figure 4). The respiration (RE) of the LG strain was 1.64 $\text{g mgO}_2 \text{ MS}^{-1} \text{ h}^{-1}$ in the control treatment; from 20 to 60 μM nitrate, the respiration rate decreased and at 80 μM nitrate it increased, reaching the highest value in 100 μM nitrate (Figure 4). The respiration of the BR strain was the highest in 60 μM nitrate and the lowest in 100 μM nitrate (Figure 4). The BR strain showed higher P than the LG strain in the control and the treatments with 60 and 100 μM nitrate and higher RE in the presence of 60 μM nitrate. The LG strain showed a higher RE than the BR strain in the treatment with 100 μM nitrate.

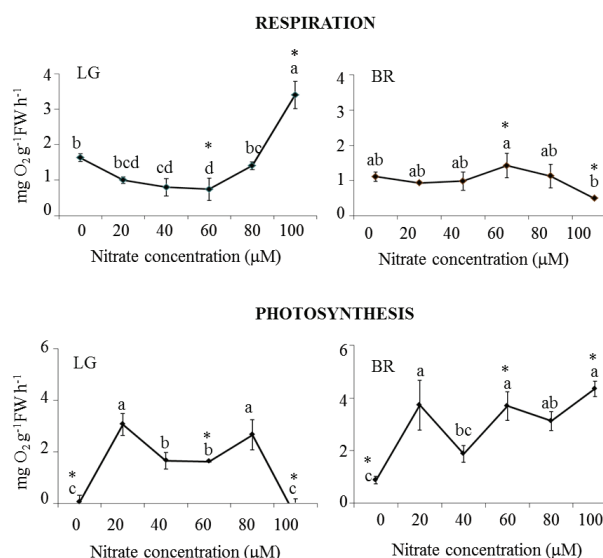


Figure 4. Respiration and photosynthesis of the light green (LG) and brown (BR) strains of *Hypnea musciformis* cultured for 28 days in sterile seawater enriched with different concentrations of nitrate. Each data point is the mean of three replicates and bars are the standard deviation. Treatments with distinct letters indicate significant differences according to the one-way ANOVA, and Tukey's multiple comparison test ($p < 0.05$). *Indicates significant differences between strains, according to the comparison test of Student-Newman-Keuls.

For the LG strain, there was significant positive correlation between the nitrate concentration and GR, protein content and RE (Table 1A). For the BR strain, there was a significant positive correlation between the nitrate concentration and GR, protein content, PC and PE contents, and P (Table 1 B). There was negative correlation between nitrate concentration and PE and Cl *a* contents for the LG strain (Table 1A).

Effects of nitrate and phosphate (N:P) availabilities

The growth rates of the LG and BR strains were lower in the control, but with no significant differences between the various treatments with nitrate plus

Table 1. Pearson correlation analysis of the variables studied for the light green (A) and brown (B) strains of *Hypnea musciformis* cultured for 28 days in sterile seawater enriched with different nitrate concentrations.

A								
Variables	GR	Protein	APC	PC	PE	Cl <i>a</i>	P	RE
[Nitrate]	*0.694	*0.602	-0.101	0.115	*-0.629	*-.557	-0.303	*0.525
	*0.001	*0.008	0.691	0.649	*0.005	*0.016	0.222	*0.025
B								
Variables	GR	Protein	APC	PC	PE	Cl <i>a</i>	P	RE
[Nitrate]	*0.506	*0.589	0.401	*0.666	*0.733	0.441	*0.7392	-0.300
	*0.032	*0.010	0.099	*0.002	*<0.001	0.066	*<0.001	0.227

GR: Growth rate; APC: Allophycocyanin; PC: Phycocyanin; PE: Phycoerythrin; Cl *a*: Chlorophyll *a*; P: Photosynthesis; RE: Respiration. In each column of the table, the upper value corresponds to the correlation coefficient and the lower value to the corresponding p value. *Significant correlation.

phosphate (Figure 5) or between the two strains.

The total soluble protein contents of the two strains were lower in the control and increased upon addition of nitrate plus phosphate (Figure 6). There were no significant differences between the two strains.

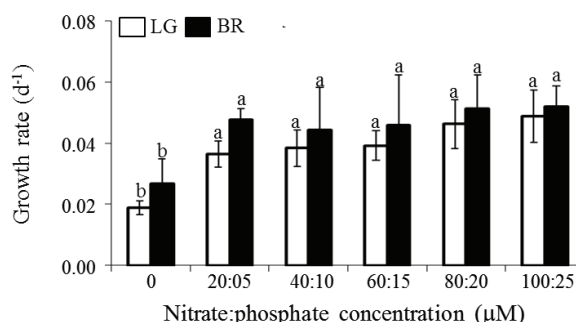


Figure 5. Growth rates of the light green (LG) and brown (BR) strains of *Hypnea musciformis* cultured for 28 days in sterile seawater enriched with different concentrations of nitrate and phosphate. Each data point is the mean of three replicates and bars are the standard deviation. Treatments with distinct letters indicate significant differences according to the one-way ANOVA, and Tukey's multiple comparison test ($p < 0.05$). *Indicates significant differences between strains, according to the comparison test of Student-Newman-Keuls.

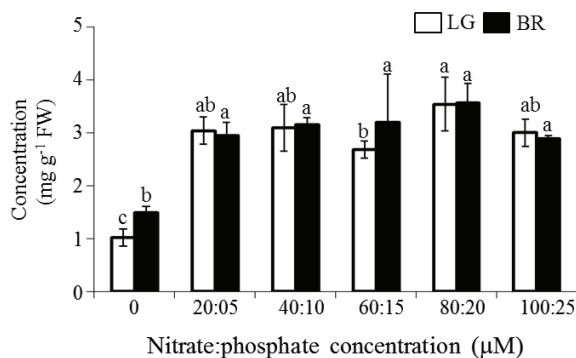


Figure 6. Concentrations (mg g^{-1} fresh weight) of soluble proteins in the light green (LG) and brown (BR) strains of *Hypnea musciformis* cultured for 28 days in sterile seawater enriched with different concentrations of nitrate and phosphate. Each data point is the mean of three replicates and bars are the standard deviation. Treatments with distinct letters indicate significant differences according to the one-way ANOVA, and Tukey's multiple comparison test ($p < 0.05$). *Indicates significant differences between strains, according to the comparison test of Student-Newman-Keuls.

The PC and APC contents of the LG strain were higher in the treatments with N:P of 20:5, 80:20 and 100:25 μM and lower in the control and for N:P of 60:15 μM (Figure 7). The contents of PC and APC of the BR strain were higher than the control in the presence of nitrate and phosphate, but with no significant differences between the treatments with these nutrients (Figure 7). The LG strain had more PC and APC than the BR strain in the control and in the treatments with N:P of 20:5 and

80:20 μM .

The LG strain presented PE only in the control treatment, whereas for BR strain the PE content was lower in the control and the treatment with N:P of 20:5 μM (Figure 7). The BR strain had more PE than the LG strain in all treatments.

For the LG strain, respiration increased from the control to a N:P of 40:10 μM , with the minimum values for treatments with N:P of 60:15 and 80:20 μM and a maximum with a N:P of 100:25 μM (Figure 8). Due to the high respiration rate, net photosynthesis was very low, being zero in the control and the treatment with N:P of 20:5, 40:10 and 100:25 μM . The highest P occurred at a N:P of 60:15 μM (Figure 8).

For the BR strain, respiration was higher in the treatment with N:P of 40:10 μM and there were no significant differences between the other treatments (Figure 8). P was also very low, with the highest values in the control and a N:P of 80:20 μM and the lowest at N:P values of 40:10 and 60:15 μM .

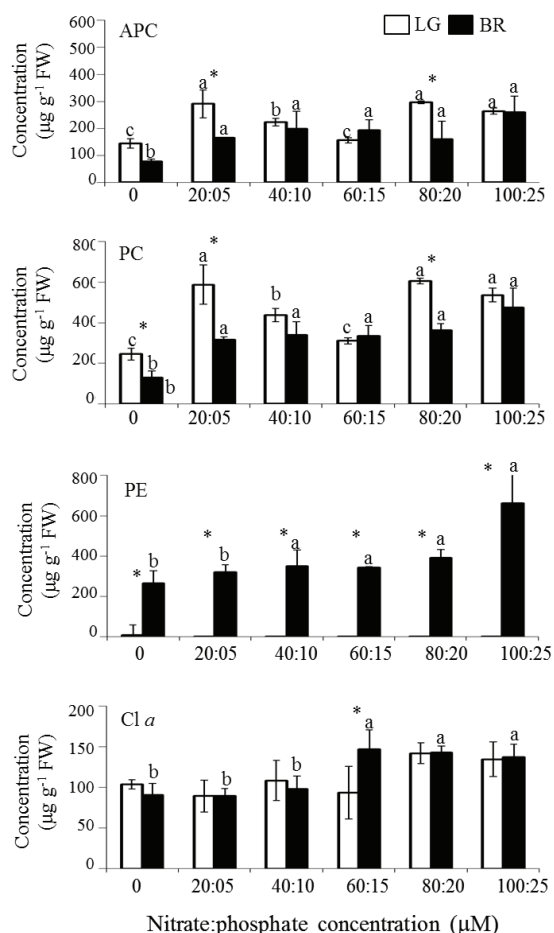


Figure 7. Concentrations ($\mu\text{g g}^{-1}$ fresh weight) of light-harvesting pigments in the light green (LG) and brown (BR) strains of *Hypnea musciformis* cultured for 28 days in sterile seawater enriched with different concentrations of nitrate and phosphate. Each data point is the mean of three replicates

and bars are the standard deviation. Treatments with distinct letters indicate significant differences according to the one-way ANOVA, and Tukey's multiple comparison test ($p < 0.05$). *Indicates significant differences between strains, according to the comparison test of Student-Newman-Keuls.

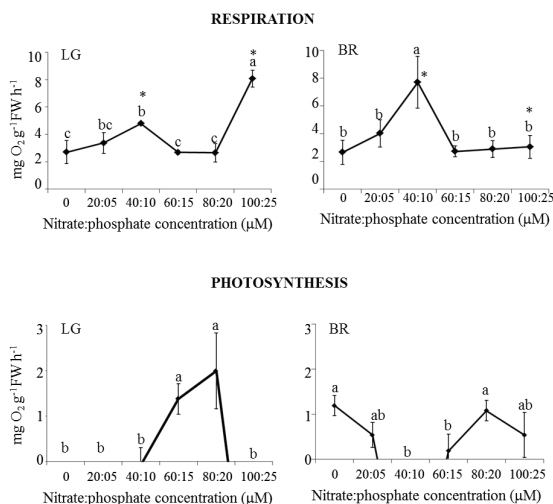


Figure 8. Respiration and net photosynthesis of the light green (LG) and brown (BR) strains of *Hypnea musciformis* cultured for 28 days in sterile seawater enriched with different concentrations of nitrate and phosphate. Each data point is the mean of three replicates and bars are the standard deviation. Treatments with distinct letters indicate significant differences according to the one-way ANOVA, and Tukey's multiple comparison test ($p < 0.05$). *Indicates significant differences between strains, according to the comparison test of Student-Newman-Keuls.

Comparing the responses of the two strains, the LG strain showed a higher P than the BR strain in the treatment with N:P of 60:15 μM , while the BR strain showed higher P than the LG strain in the control. The LG strain showed a higher RE than the BR strain at a N:P of 100:25 μM , while the BR strain showed a higher RE than the LG strain at a N:P of 40:10 μM .

For the LG strain, there was a significant positive correlation between the nitrate: phosphate concentrations and protein, APC, PC, Cl *a*, P and RE

(Table 2A). For the BR strain, there was significant positive correlation between the nitrate: phosphate concentrations and GR, protein, APC, PC, PE and Cl *a* (Table 2B).

Comparison of different variables between experiments with nitrate addition and with addition of nitrate plus phosphate (N:P)

There were no significant differences in the GR of the two strains of *H. musciformis* cultured in medium with nitrate or with N:P additions. The LG strain showed higher protein and APC contents in the treatment with 80:20 μM N:P in comparison to the medium without phosphate enrichment.

When there were significant differences between the treatments with nitrate or N:P addition, pigments and respiration of the two strains were higher in the treatments with N:P addition, while photosynthesis of the two strains was significantly higher in the treatments with nitrate alone.

Discussion

Effects of nitrate availability

The growth rates of the LG and BR strains of *Hypnea musciformis* were higher in the range from 20 to 100 μM nitrate. Growth rates of *Laminaria saccharina* (L.) J.V. Lamour reached their maximum value at lower concentrations of nitrate, between 5 and 10 μM (Wheeler & Weidner, 1983).

The LG strain of *H. musciformis* produced PE only in the control treatment; with nitrate addition to the medium, the concentration of this pigment was below the limit of detection. The optimum nitrate concentration for production of APC, PC, Cl *a* and protein in the LG strain and for phycobiliproteins and protein in the BR strain was 20 μM nitrate. Andria et al. (1999) reported that the growth rate of *Gracilaria* sp. was higher in treatments with low N availability

Table 2. Pearson correlation analysis of the variables studied for the light green (A) and brown (B) strains of *Hypnea musciformis* cultured for 28 days in sterile seawater enriched with different nitrate and phosphate concentrations.

A								
Variables	GR	Protein	APC	PC	PE	Cl <i>a</i>	P	RE
[Nitrate and phosphate]	*0.820 * < 0.001	*0.633 *0.004	*0.544 *0.029	*0.591 *0.015	-0.482 0.058	*0.552 *0.017	-0.257 0.303	*0.548 *0.018
B								
Variables	GR	Protein	APC	PC	PE	Cl <i>a</i>	P	RE
[Nitrate and phosphate]	*0.478 *0.044	*0.587 *0.010	*0.563 *0.035	*0.796 * < 0.001	*0.707 *0.004	*0.773 * < 0.001	0.063 0.801	-0.159 0.530

GR: Growth rate; APC: Allophycocyanin; PC: Phycocyanin; PE: Phycoerythrin; Cl *a*: Chlorophyll *a*; P: Photosynthesis; RE: Respiration. In each column of the table, the upper value corresponds to the correlation coefficient and the lower value to the corresponding *p* value. *Significant correlation.

than in treatments with high N availability. However, the soluble protein and PE contents decreased when the algae was grown under low nitrogen availability, indicating the role of these compounds as nitrogen storage pools under conditions of nutrient limitation and their allocation for growth (Andria et al., 1999). The opposite response was observed for the LG strain of *H. musciformis*, which produced PE only in the control without N addition.

The optimum nitrate concentration for net photosynthesis by the LG and BR strains of *Hypnea musciformis* was 20 μM . The photosynthesis of the LG strain was completely inhibited at 100 μM , while the photosynthesis of the BR strain reached its maximum values. For *Gracilaria tenuistipitata* Chang et Xia (García-Sánchez et al, 1993) grown at low and high concentrations of nitrate (0.1 and 1 mM), the oxygen evolution remained high for 10 days during treatment with high N concentration, but ceased upon treatment with low concentrations of nitrate. A similar result was observed for the BR strain of *H. musciformis*.

Growth rates, protein content and respiration of the LG strain of *H. musciformis* correlated positively with the increase of nitrate availability, while the nitrate concentration correlated positively with the growth rate, protein content, PC and PE content and photosynthesis of the BR strain. For *Gracilaria foliifera* var. *angustissima* (Harvey) Taylor (Lapointe, 1981) and for the wild strain and chromatic variants of *Gracilaria birdiae* Plastino and E. C. Oliveira (Costa, 2005), the growth rates did not increase with increasing nitrate concentrations. However, Costa (2005) observed an increase in the pigment content (except for allophycocyanin) of the *G. birdiae* variants in response to an increase in nitrate availability, similar to what we found for the BR strain of *H. musciformis*.

The respiration of the LG strain of *H. musciformis* increased with increasing nitrate availability, whereas for the BR strain there was an increase in photosynthesis under these conditions. This result points to metabolic differences between the two strains in terms of the metabolism of nitrogen assimilation, indicating that the BR strain can fix more photosynthetic carbon than the LG strain. On the other hand, the LG strain can supply the demand of carbon necessary for the synthesis of aminoacids through glycolysis.

Effects of nitrate and phosphate (N:P) availabilities

The N:P ratio influenced the seaweed growth rates, but the growth rates of the LG and BR strains of *Hypnea musciformis* did not differ significantly at the different nitrate and phosphate concentrations when the N:P ratio was maintained constant at 4:1. The same

was not observed for *Gracilaria cornea* J. Agardh, for which the highest growth rates occurred for N:P ratios of 10:1 and 5:1 and the lowest growth rates occurred for ratios of 10:0 and 10:10 (Navarro-Angulo & Robledo, 1999).

For both strains of *Hypnea musciformis*, there was a positive correlation between the increase of phosphate in the medium and the content of photosynthetic pigments. However, Chopin et al. (1995) found that phosphorus had no significant effect on photosynthesis and photosynthetic pigment contents in *Chondrus crispus* Stackhouse. Moreover, phosphorus was the most important limiting nutrient for the productivity of *G. tikvahiae* (Lapointe, 1987) in the coastal waters of Florida and photosynthesis was stimulated more by phosphorus than by nitrogen. Enrichment of phosphorus also stimulated the photosynthetic rates of *Sargassum natans* (L.) and *Sargassum fluitans* Gaillon (Børgesen) Børgesen (Lapointe, 1986). However, this was not observed for the two strains of *H. musciformis*, since photosynthesis in the algae cultured with phosphate was generally lower than that in the absence of phosphate.

Comparison between the LG and BR strains of Hypnea musciformis

A comparison of the responses of the LG and BR strains of *H. musciformis* growing in different nitrate concentrations showed that the BR strain had a higher growth rate and protein content than the LG strain in several of the treatments. Costa (2005) also reported that the light green strain of *Gracilaria birdiae* showed a lower growth rate, regardless of the nitrate concentration tested, apparently related to metabolic deficiencies of this colour variant. However, the LG strain of *H. musciformis* showed higher photosynthesis than the BR strain in treatments with N:P of 40:10, 60:15 and 80:20 μM . These results indicate that the LG strain can have higher photosynthetic performance than the BR strain under certain conditions of nitrogen and phosphorus availability. When only nitrate was added to the seawater, the LG strain of *H. musciformis* stored nitrogen mainly as proteins, whereas the BR strain stored it as proteins and pigments (PC and PE). With N:P addition to the seawater, the LG strain also stored nitrogen in the form of pigments (APC, PC). These results indicate that there are differences in the nitrogen metabolism of the two colour strains of *Hypnea musciformis* and suggest an important role for phosphorus in nitrogen metabolism.

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***Correspondence**

Nair S. Yokoya
Núcleo de Pesquisas em Ficologia, Instituto de Botânica,
Secretaria de Estado do Meio Ambiente
Av. Miguel Estefano, 3687, 04301-012 São Paulo-SP, Brazil
nyokoya@pq.cnpq.br
Tel.: +55 11 5067 6121