

Intraspecific variations in colour morphs of *Hypnea musciformis* (Rhodophyta) in relation to nitrogen availability

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ABSTRACT – (Intraspecific variations in colour morphs of *Hypnea musciformis* (Rhodophyta) in relation to nitrogen availability). Effects of nitrate availability in two culture media (von Stosch (VSES), and artificial ASP 12-NTA), and nitrogen sources (seawater enriched with nitrate, ammonium or urea in concentrations ranging from zero to 30 μM) were evaluated in two brown morphs (BR-1, BR-2), one light-green morph (LG) and one dark-green morph (DG) of *Hypnea musciformis* (Wulfen) J.V. Lamour. Higher growth rates of the four morphs were observed in VSES medium. However, artificial ASP12-NTA medium induced tetrasporangium development in DG morph. Growth rates of the four colour morphs followed kinetic of saturation-type nutrient uptake in treatments with urea. In contrast, growth rates of BR-1, BR-2 and LG morphs were inversely proportional to ammonium concentrations, and those higher than 15 μM were lethal. Growth responses of colour morphs of *H. musciformis* showed intraspecific variations, and they could be used as bioindicators of nitrogen pollution in marine environment by their low tolerance to ammonium.

Key words: ammonium, *Hypnea musciformis*, nitrate, urea

RESUMO – (Variações intraespecíficas em morfos pigmentares de *Hypnea musciformis* (Rhodophyta) em relação à disponibilidade de nitrogênio). Efeitos da disponibilidade de nitrato em dois tipos de meio de cultura (meio von Stosch (VSES) e meio artificial ASP 12-NTA), e fonte de nitrogênio (água do mar enriquecida com nitrato, amônio e uréia em concentrações de zero a 30 μM) foram avaliados em dois morfos marrons (BR-1, BR-2), um morfo verde-claro (LG) e um morfo verde-escuro (DG) de *Hypnea musciformis* (Wulfen) J.V. Lamour. As maiores taxas de crescimento dos quatro morfos foram observadas em meio VSES. Entretanto, o meio artificial ASP12-NTA induziu o desenvolvimento de tetrasporângios no morfo DG. As taxas de crescimento dos quatro morfos variaram segundo uma cinética de saturação nos tratamentos contendo uréia. Por outro lado, as taxas de crescimento dos morfos BR-1, BR-2 e LG foram inversamente proporcionais às concentrações de amônio, e aquelas maiores do que 15 μM foram letais. As respostas em crescimento dos quatro morfos de *H. musciformis* apresentaram variações intraespecíficas, e estes morfos podem ser bioindicadores de poluição por nitrogênio em ambientes marinhos devido à sua baixa tolerância ao amônio.

Palavras-chave: amônio, *Hypnea musciformis*, nitrato, uréia

Introduction

The genus *Hypnea* (Gigartinales, Rhodophyta) includes 50 species distributed in warm waters (Masuda *et al.* 1997), much of them with economic importance in several countries (Critchley & Ohno 1998). In Brazil, *Hypnea musciformis* (Wulfen) J.V. Lamour. has a wide geographical distribution, and is the main raw material for carrageenan production (Berchez *et al.* 1993).

Along the Brazilian coast, populations of *Hypnea musciformis* usually have a brown phenotype. However, one population composed by brown and

dark-green specimens was found in Espírito Santo State. Besides, a brown plant with only one green branch was collected, and a phycoerythrin-deficient morph (light-green phenotype) was originated from this green branch. In laboratory, these specimens were isolated, and cultured in different environmental conditions as irradiance levels, photoperiod, temperature and salinity in order to evaluate if the thallus colour could be an acclimation to environmental variations. The colour of these *H. musciformis* morphs is a stable characteristic, and is not a result of photoacclimation processes (Yokoya *et al.* 2003, Martins *et al.* 2008). The occurrence of

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colour variants in Brazilian red algae was reported to some *Gracilaria* species, as *G. birdiae* Plastino & E.C. Oliveira, *G. cornea* J. Agardh, and *G. domingensis* (Kützting) Sonder *ex* Dickie (Plastino 2003). The first report on colour variants of *Hypnea musciformis* was described by Yokoya *et al.* (2003), and the light-green morph was characterized by having lower phycoerythrin concentration, and higher phycocyanin and allophycocyanin concentrations than the brown morph.

The major nitrogen storage pools in macroalgae are amino acids and proteins, and in nitrogen-limited condition, phycoerythrin is an important source of nitrogen (Bird *et al.* 1982). Then, the colour morphs of *H. musciformis* could be a good experimental systems to study different processes of nitrogen metabolism.

Several approaches on nitrogen metabolism in macroalgae were studied as the effects of nitrogen sources and concentrations (Hanisak 1983, 1990, García-Sánchez *et al.* 1993, Navarro-Angulo & Robledo 1999, Pedersen *et al.* 2004), nitrogen uptake, assimilation and storage (D'Elia & Deboer 1978, Fujita 1985, Hwang *et al.* 1987, Andria *et al.* 1999, Taylor & Rees 1999, Naldi & Wheeler 2002, Collén *et al.* 2004, Martínez & Rico 2004, Phillips & Hurd 2004), and the regulation of nitrate reductase activity (Wheeler & Weidner 1983, Hurd *et al.* 1995, Lopes *et al.* 2002, Granbom *et al.* 2004). Few studies on nitrogen metabolism were conducted with Brazilian macroalgae, except for the uptake of different nitrogen sources in the brown alga *Laminaria abyssalis* A.B. Joly & E.C. Oliveira (Braga & Yoneshigue-Valentin 1994, 1996), the regulation of nitrate reductase activity in red algae *Gracilariopsis tenuifrons* (C.J. Bird & E.C. Oliveira) Fredericq & Hommers (Rossa 1999), *Gracilaria birdiae* (Donato 2005) and *G. caudata* J. Agardh (Chow *et al.* 2007), and the effects of nitrate availability in *G. birdiae* (Costa 2005).

The objectives of our study were to determine the effects of nitrate concentration in different culture media as well as to evaluate different nitrogen sources and concentrations on growth of colour morphs of *Hypnea musciformis*.

Materials and methods

Unialgal cultures - Four colour tetrasporophytes of *Hypnea musciformis* (Wulfen) J.V. Lamour. were selected: two brown morphs (BR-1 and BR-2), one light-green morph (LG) and one dark-green morph

(DG). The LG morph was a phycoerythrin-deficient mutant, and was originated from a green branch, which had arisen probably as a spontaneous mutation in a brown plant (BR-2) collected from Ponta da Baleia, Espírito Santo, Brazil; both morphs were originated by vegetative propagation in laboratory. BR-1 and DG morphs were originated from carpospores liberated, respectively, by brown and dark-green plants collected from Praia de Formosa, Espírito Santo, Brazil. Voucher specimens were deposited in SP herbarium, with the following accession numbers: SP 365645 (LG morph), SP 365646 (BR-2 morph), SP 365671 (BR-1 morph) and SP 365672 (DG morph).

The colour morphs of *H. musciformis* were cultured in sterilized seawater (salinity of 30-32 psu) enriched with von Stosch's solution at half strength (VSES/2, with 250 μM N-nitrate) following Edwards (1970) and modified by Yokoya (2000), with vitamin concentrations reduced to 50%. Medium renewal was carried out weekly. Cultures were incubated under 23 ± 1 °C, irradiance of 30.0-50.0 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$, provided by cool-white fluorescent lamps with 14:10h light:dark cycle, without aeration.

Nitrogen experiments - Effects of nitrate availability were tested in different culture media: sterilized seawater enriched with von Stosch's solution (VSES), and artificial ASP 12-NTA medium, following Iwasaki (1961), and modified by Yokoya (2000), with different nitrate concentrations (125, 250 and 500 μM) for 28 days. Both culture media presented salinity of 30 psu and pH of 8.0.

Effects of nitrogen sources and concentrations: two inorganic nitrogen sources (sodium nitrate and ammonium chloride) and one organic source (urea) were added to sterilized seawater in concentrations of zero, 5, 10, 15, 20, 25 and 30 μM . Low concentrations of ammonium (zero to 5 μM) were tested in a second experiment to determine the optimal concentrations for growth, as colour morphs did not tolerate higher concentrations of ammonium. Phosphorus was added ($\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$) in concentrations to maintain the N:P ratio of 4:1. All treatments were prepared with sterilized seawater provided by only one collection, and experimental period was 21 days.

Other experimental conditions were the same as described for unialgal cultures. Each treatment was tested with three replicates, with four apical segments of 10 mm, cultured in 250 mL Erlenmeyer flasks with 150 mL of medium. Fresh biomass was recorded weekly in the same intervals of medium renewal.

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

Data analysis - Data were analyzed by one-way and two-way analyses of variance (ANOVA) and, when normality and equal variance tests did not pass, the data were analyzed by ANOVA on Ranks. Student-Newman-Keuls post hoc test was conducted to distinguish significantly different results ($p < 0.05$) following the ANOVA tests. Statistical tests were performed by SigmaStat software (version 1.0).

Results

Nitrate availability in VSES and ASP 12-NTA media - The highest growth rates of the four morphs were observed in VSES medium. However, differences among treatments (nitrate concentrations ranging from 125 to 500 μM) were not significant, except for BR-2 morph, which showed the highest growth rate at low nitrate concentration (figure 1). In ASP 12-NTA medium, growth rates of the four morphs were lower than those observed in VSES medium, and the highest growth rates of the BR-1 and DG morphs were observed in low nitrate concentration (125 μM) (figure 1). A comparison among the four morphs showed intraspecific variations to the response to nitrate availability (table 1), and DG and LG morphs showed, respectively, the lowest and the highest growth rates in both culture media (figure 2).

The ASP 12-NTA medium stimulated the development of tetrasporangia in DG morph (figure 3A), and specimens cultured under 250 and 125 μM of nitrate became fertile after four and seven weeks, respectively. Some tetraspores germinated “in situ” and tetrasporelings grew on the stichidium (figure 3B), or tetraspores, which were settled onto coverslips placed on the bottom of culture flasks,

germinated and gave rise to several tetrasporelings (figure 3C-G).

Effects of nitrogen sources and concentrations - Growth rates of BR-1, BR-2 and LG morphs of *Hypnea musciformis* cultured with different concentrations of N-nitrate did not vary significantly when compared to the control (figure 4). However, growth rates of DG morph increased significantly under treatments with N-nitrate concentrations from 5 to 30 μM (figure 4).

Variations of growth rates on the studied morphs with the addition of zero to 30 μM of N-ammonium were significantly different (figure 5), except for the DG morph. BR-1, BR-2 and LG morphs showed inverse relationship between growth rates and N-ammonium concentrations, and concentrations equal and higher than 15 μM were lethal for LG and BR-2 morphs, respectively.

The experiment with low concentrations of N-ammonium (from zero to 5 μM) showed that only green morphs presented significant differences on growth rates when compared to the control (figure 6). Optimum concentration for growth of the DG morph was 1,0 μM N-ammonium while LG morph showed higher growth rates at 1.0 and 4.0 μM N-ammonium (figure 6).

Growth rates of the four colour morphs in relation to the increase of N-urea concentration followed kinetic of saturation-type nutrient uptake (figure 7). Growth rates of BR-1 and DG morphs increased at concentrations higher than 5 μM N-urea, and the optimal concentrations for growth were 10 and 5 μM N-urea, respectively (figure 7).

A comparison among the four morphs indicated that responses to different nitrogen concentrations (nitrate, ammonium, and urea) as well as nitrogen sources (ammonium and urea) showed intraspecific variations (table 2). Besides, the interaction between nitrogen concentrations and sources had significant effects on colour morphs of *H. musciformis* (table 3).

Table 1. Bifactorial analysis based on growth rates of colour morphs (BR-1, BR-2, DG, and LG) of *Hypnea musciformis* cultured in von Stosch and ASP 12-NTA media with different concentrations of N-nitrate. Variables: N-nitrate concentrations (A), and morphs (B). Bold letters/numbers indicate significant effects.

Medium	Effects	Degrees of freedom	F	P
Von Stosch	A	2	2.81	0.0802
	B	3	73.27	<0.0001
	Interaction A/B	6	1.27	0.3082
ASP 12-NTA	A	2	62.40	<0.0001
	B	3	99.56	<0.0001
	Interaction A/B	6	8.03	<0.0001

Table 2. Bifactorial analysis based on growth rates of colour morphs (BR-1, BR-2, DG, and LG) of *Hypnea musciformis* cultured in sterilized seawater with addition of different concentrations of N-nitrate, N-ammonium and N-urea. Variables: Nitrogen concentrations (A), morphs (B). Bold letters/numbers indicate significant effects.

Nitrogen sources (concentrations)	Effects	Degrees of freedom	<i>F</i>	P
Nitrate (0, 5,...30 µM)	A	6	2.60	0.0289
	B	4	1.87	0.1311
	Interaction A/B	24	2.07	0.0155
Ammonium (0, 5,...30 µM)	A	6	16.09	<0.0001
	B	4	9.57	<0.0001
	Interaction A/B	24	1.70	0.0583
Ammonium (0, 1,...5 µM)	A	6	8.16	<0.0001
	B	4	1.80	0.1605
	Interaction A/B	24	2.15	0.0232
Urea (0, 5,...30 µM)	A	6	10.78	<0.0001
	B	4	4.28	0.0048
	Interaction A/B	24	1.88	0.0307

Table 3. Bifactorial analysis based on growth rates of colour morphs (BR-1, BR-2, DG, and LG) of *Hypnea musciformis* cultured in sterilized seawater with addition of different concentrations of N-nitrate, N-ammonium and N-urea. Variables: Nitrogen concentrations (A), nitrogen sources (B). Bold letters/numbers indicate significant effects.

Morphs	Effects	Degrees of freedom	<i>F</i>	P
BR-1	A	6	2.27	0.0545
	B	2	85.52	<0.0001
	Interaction A/B	12	6.28	<0.0001
DG	A	6	8.39	<0.0001
	B	2	178.98	<0.0001
	Interaction A/B	12	5.21	<0.0001
BR-2	A	6		0.5532
	B	2	0.830	<0.0001
	Interaction A/B	12		0.0016
LG	A	6	2.52	0.0355
	B	2	105.97	<0.0001
	Interaction A/B	12	3.47	0.0013

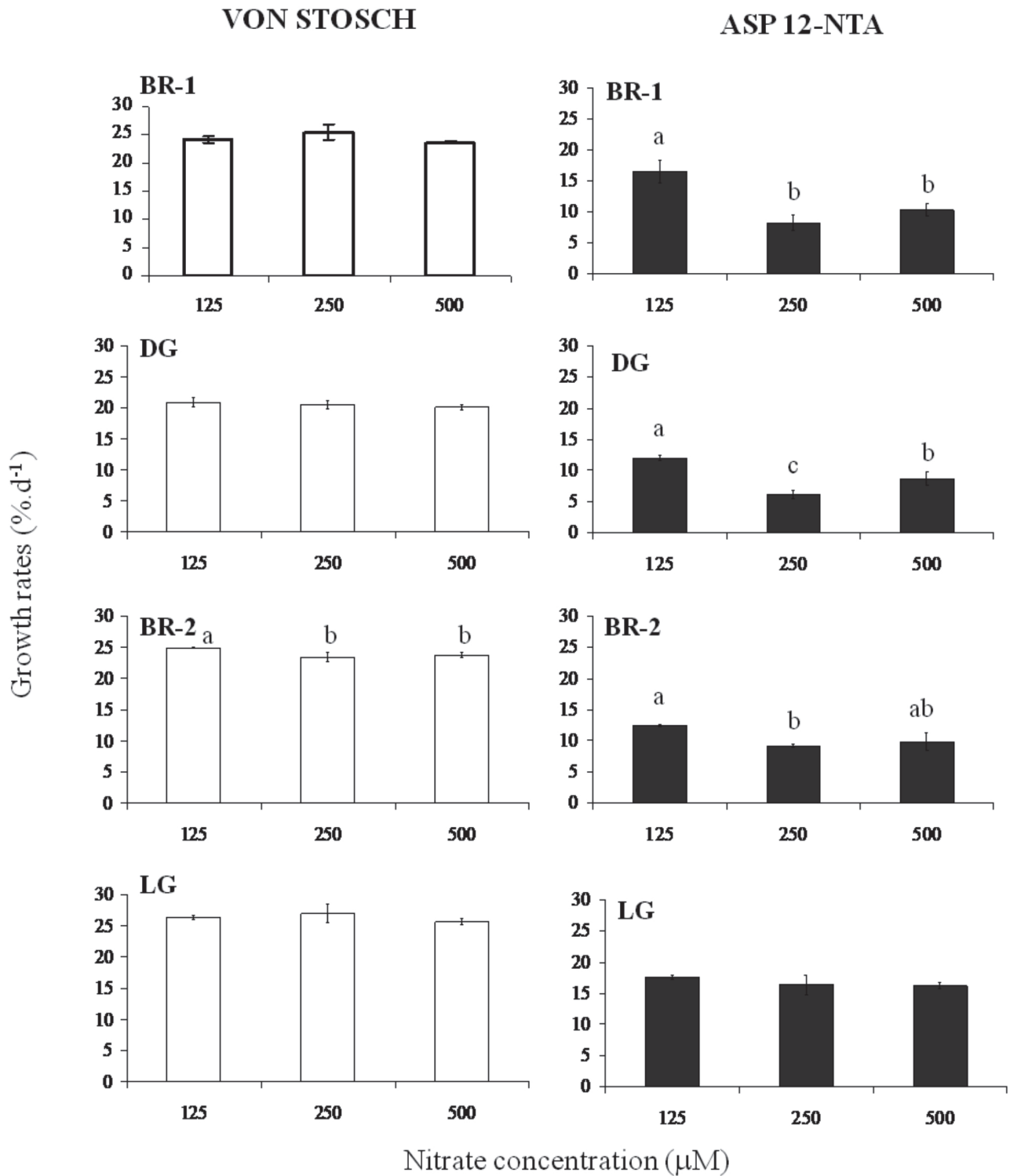


Figure 1. Growth rates of brown morphs (BR-1 and BR-2), dark-green morph (DG) and light-green morph (LG) of *Hypnea musciformis* cultured at different concentrations of N-nitrate in Von Stosch and ASP 12-NTA media. Each data point represents the mean ($n = 3$, \pm SD). Treatments marked by different letters are significantly different according to ANOVA and Student-Newman-Keuls comparison test ($p < 0.05$). To BR-2 and LG morphs the data were analyzed by ANOVA on Ranks.

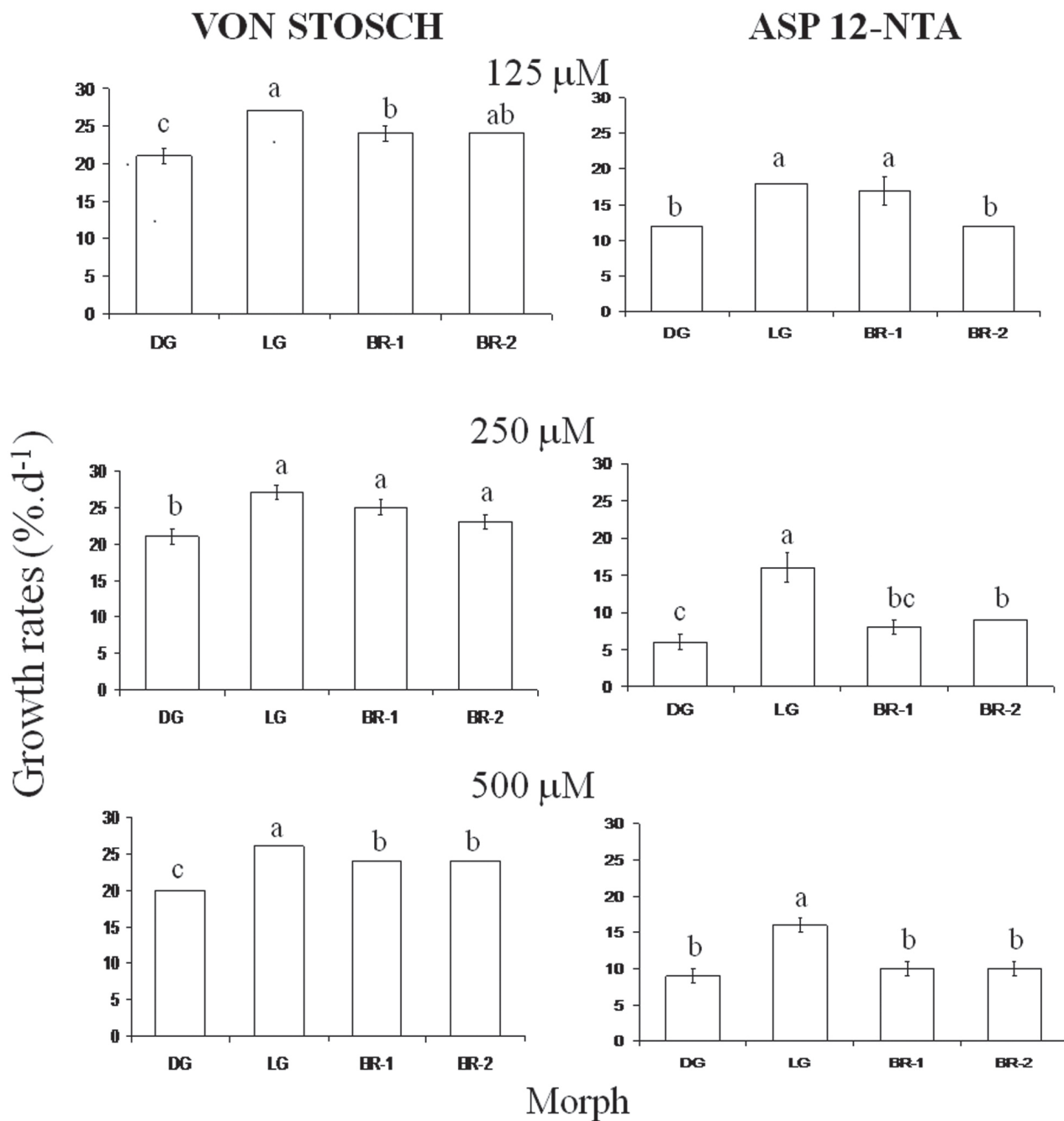


Figure 2. Growth rates of the four morphs of *H. musciformis* (brown morphs, BR-1, BR-2, dark-green morph, DG, and light-green morph, LG) cultured at different concentrations of N-nitrate in Von Stosch and ASP 12-NTA media. Each data point represents the mean ($n = 3, \pm \text{SD}$). Treatments marked by different letters are significantly different according to ANOVA and Student-Newman-Keuls comparison test ($p < 0.05$).

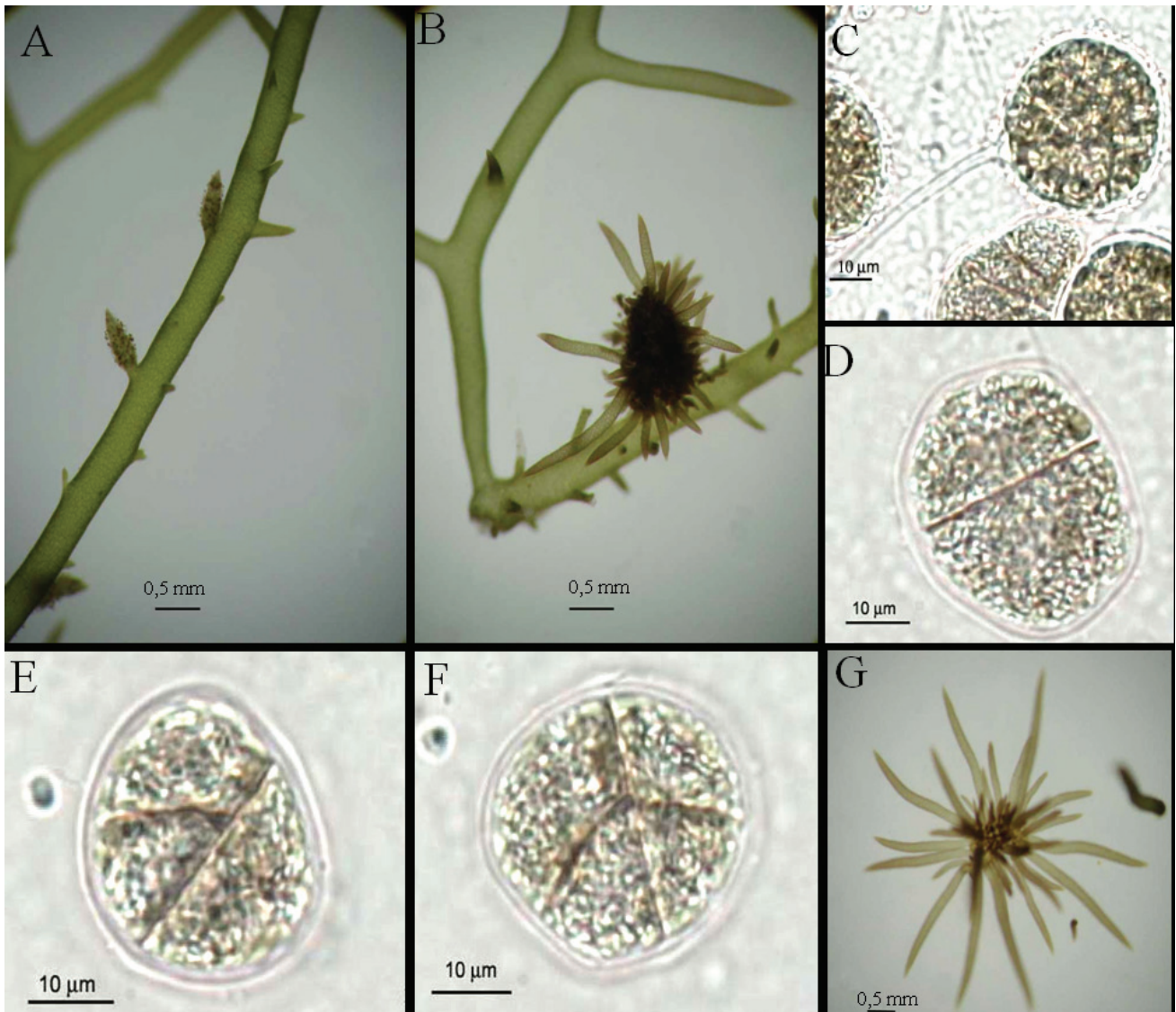


Figure 3. Dark green morph (DG) of *H. musciformis* cultured at ASP 12-NTA medium with 250 μM N-nitrate. A. branch with stichidia; B. germination “in situ”, and tetrasporelings growing on mother-plant; C-G. process of tetraspore germination and tetrasporeling development: C. tetrasporeling with hialin hair, D. two-celled tetrasporeling; E. three-celled tetrasporeling; F. four-celled tetrasporeling; G. tetrasporelings after two weeks.

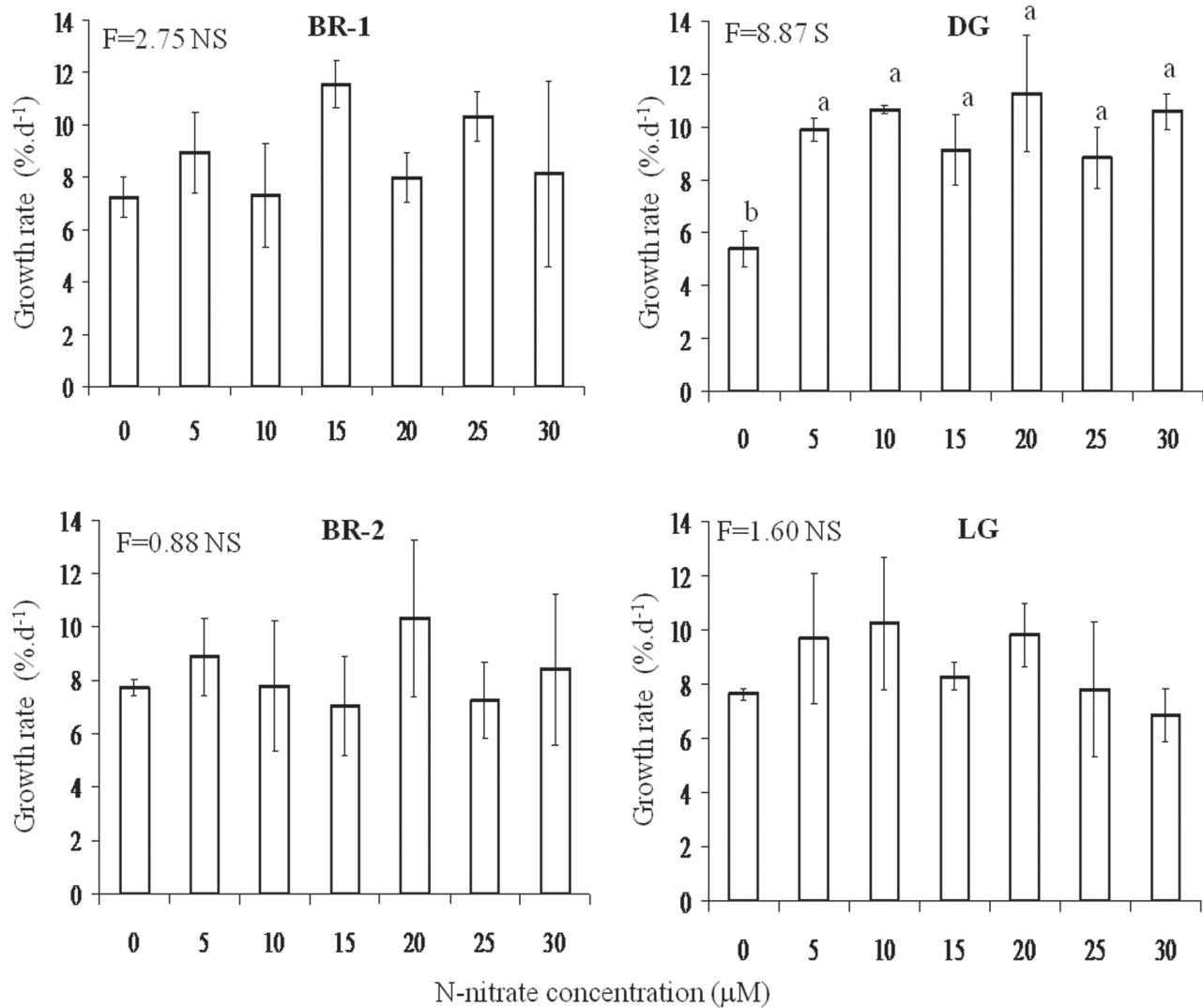


Figure 4. Growth rates of brown morphs (BR-1 and BR-2), dark-green morph (DG) and light-green morph (LG) of *H. musciformis* cultured at seven different concentrations of N-nitrate. Each data point represents the mean ($n = 3$, \pm SD). Treatments marked by different letters are significantly different according to ANOVA and Student-Newman-Keuls comparison test ($p < 0.05$).

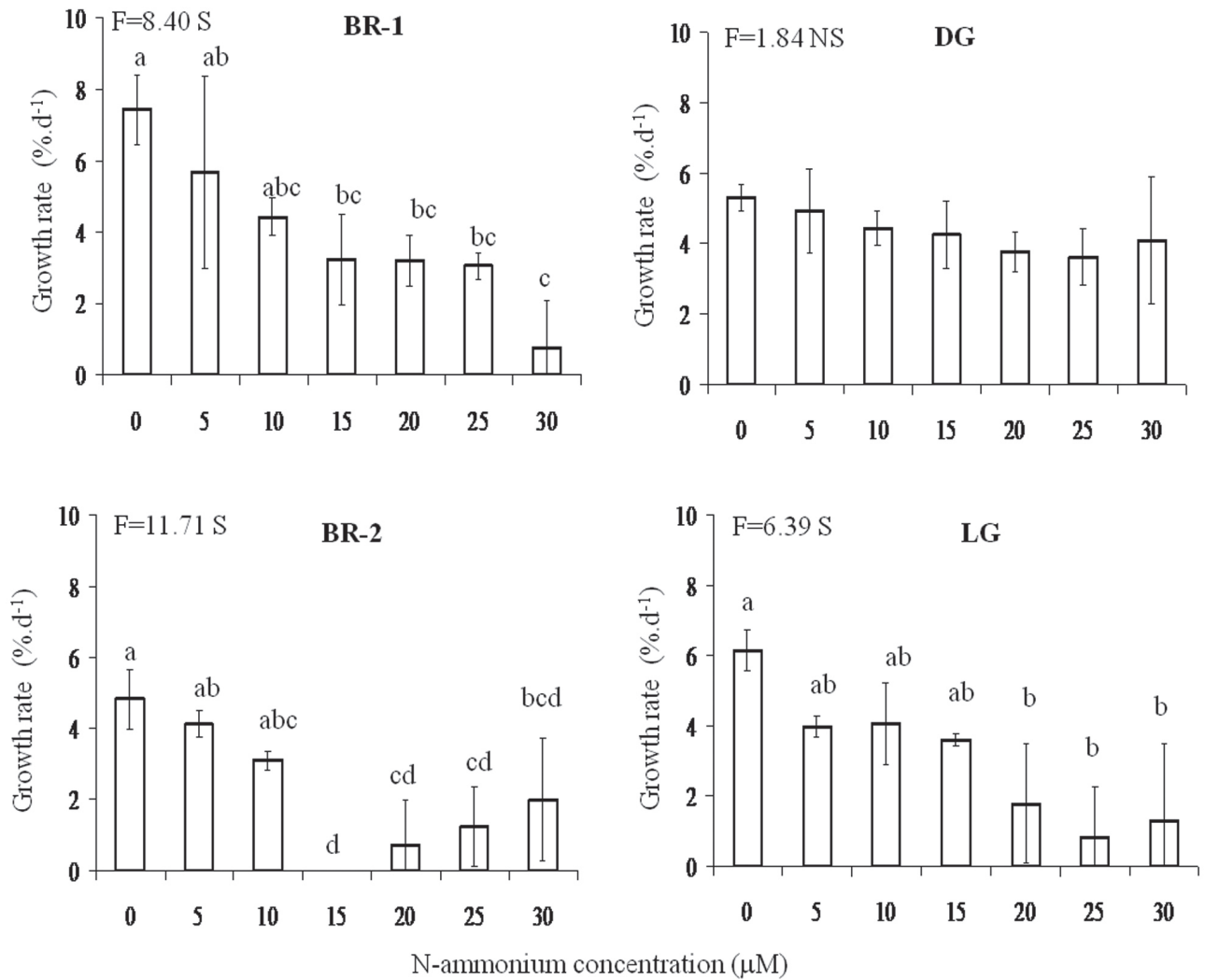


Figure 5. Growth rates of brown morphs (BR-1 and BR-2), dark-green morph (DG) and light-green morph (LG) of *H. musciformis* cultured at seven different concentrations of N-ammonium. Each data point represents the mean ($n = 3$, \pm SD). Treatments marked by different letters are significantly different according to the ANOVA and Student-Newman-Keuls comparison test ($p < 0.05$). To BR-1 morph the data were analyzed by ANOVA on Ranks.

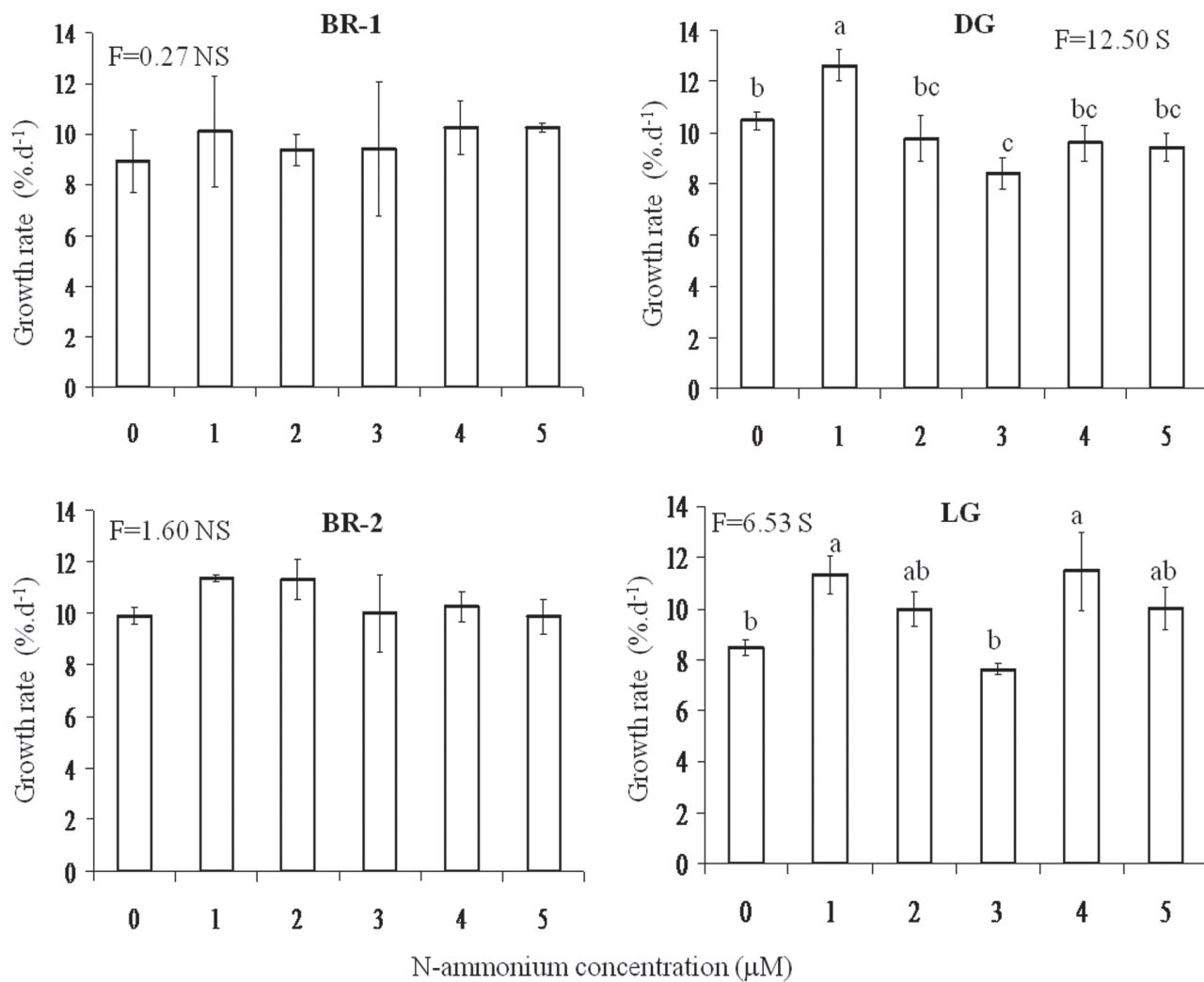


Figure 6. Growth rates of brown morphs (BR-1 and BR-2), dark-green morph (DG) and light-green morph (LG) of *H. musciformis* cultured at different concentrations of N-ammonium. Each data point represents the mean ($n = 3$, \pm SD). Treatments marked by the different letter are significantly different according to ANOVA and Student-Newman-Keuls comparison test ($p < 0.05$). To DG morph the data were analyzed by ANOVA on Ranks.

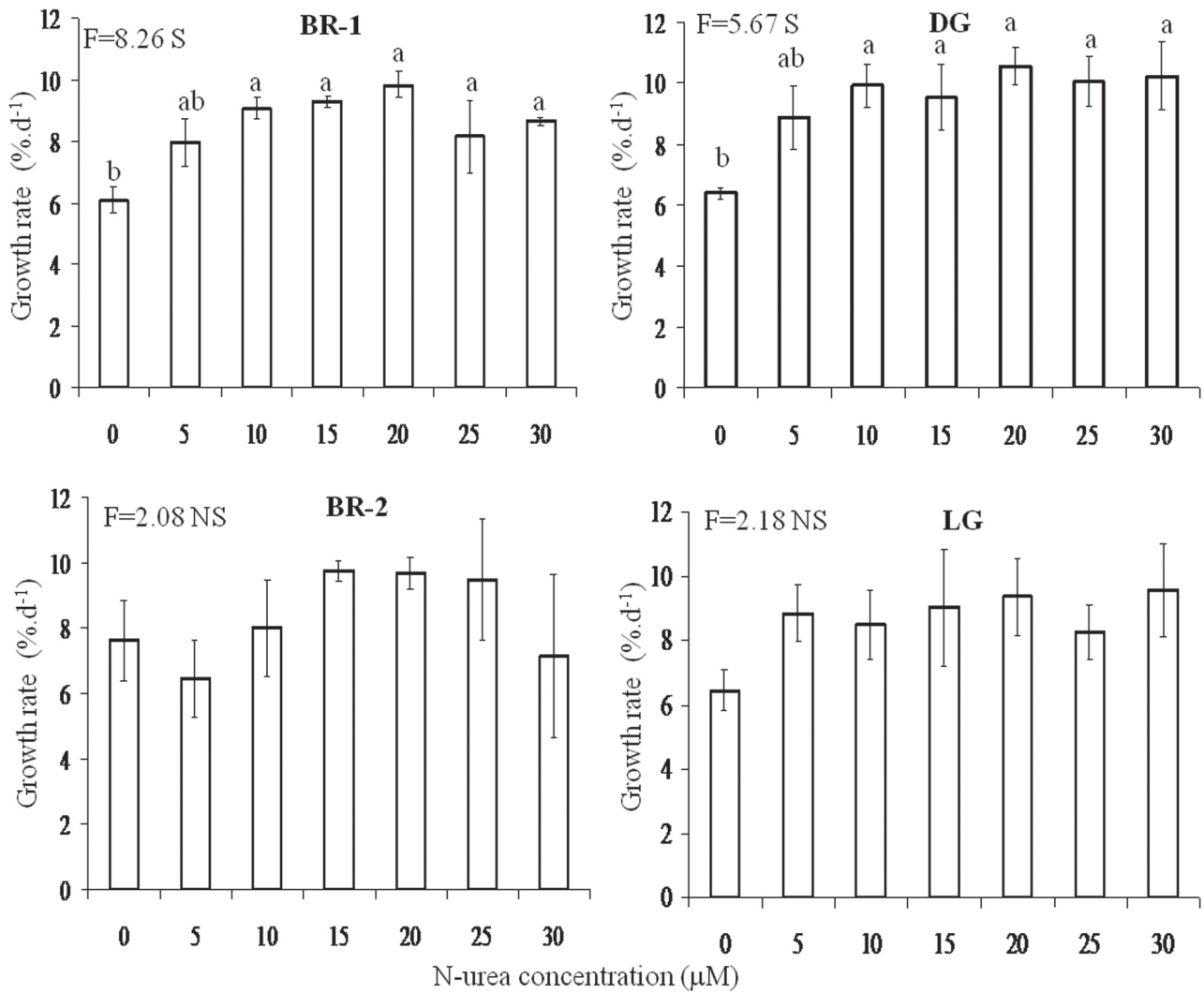


Figure 7. Growth rates of brown morphs (BR-1 and BR-2), dark-green morph (DG) and light-green morph (LG) of *H. musciformis* cultured at different concentrations of N-urea. Each data point represents the mean ($n = 3$, \pm SD). Treatments marked by different letters are significantly different according to the ANOVA and Student-Newman-Keuls comparison test ($p < 0.05$).

Discussion

Seaweeds are able to use different forms of nitrogen, and each species could have ability to use specific nitrogen sources (Hanisak 1983, Lobban & Harrison 1994). Considering seaweed growth, three patterns could be observed: 1. higher growth with nitrate, 2. higher growth with ammonium, and 3. similar growth with nitrate and ammonium (Hanisak 1990). Besides these patterns, some species showed higher growth with addition of organic nitrogen (urea) instead of inorganic nitrogen sources, as observed in the red algae *Pterocladia capillacea* (S.G. Gmel.) Santel. & Hommers. (cited as *Pterocladia capillacea*, Nasr *et al.* 1968) and the green algae *Ulva fasciata* Delile (Mohsen *et al.* 1974). The red alga *Gracilaria cornea* did not have preference between organic and inorganic nitrogen sources (Navarro-Angulo & Robledo 1999).

The four colour morphs of *Hypnea musciformis* showed higher growth rates with addition of nitrate and urea. However, ammonium inhibited the growth of BR-1, BR-2 and LG morphs, and concentrations higher than 15 μM were lethal to LG and BR-2 morphs. At high concentrations, ammonium could be toxic for some seaweeds (Waite & Mitchell 1972, Prince 1974), and the four morphs of *H. musciformis* showed similar responses. These results could be explained by the coupling of nitrogen metabolism and photosynthetic pathways (Turpin 1991), and the increase in intracellular ammonium concentration needs a higher carbon skeleton availability for nitrogen assimilation. Probably, intracellular ammonium could not be assimilated by *H. musciformis* colour morphs, and its accumulation into the cytoplasm decreased the internal pH, what was prejudicial to the cells (Turpin 1991). In contrast, different results were observed by Haines (1976) in a cultivation system, where *H. musciformis* showed higher growth in effluent enriched with ammonium than with nitrate, and ammonium uptake was faster than nitrate uptake (Haines & Wheeler 1978).

A comparison among the four morphs showed intraspecific variations in relation to nitrate availability in VSES and ASP 12-NTA media; DG and LG morphs showed, respectively, the lowest and the highest growth rates in both culture media. Then, DG morph showed lower growth rates than the BR-1 morph, and the best performance of wild specimens was also observed in the red specimens of *Gracilaria birdiae* (cited as *Gracilaria* sp. by Ursi & Plastino

2001), and in brown specimens of *Kappaphycus alvarezii* (Doty) Doty *ex* Silva (Paula *et al.* 1999). However, different results were observed in BR-2 and LG morphs, since the LG morph (phycoerythrin-deficient mutant) had higher growth rate than the BR-2 morph (wild specimen). Similar results were observed for a green variant of *Kappaphycus striatum* (Schmitz) Doty (Gerung & Ohno 1997), and for yellowish-brown mutant of *Porphyra yezoensis* Ueda, which had higher growth than the wild specimen and other colour mutants (Yan *et al.* 2000).

In the four colour morphs of *Hypnea musciformis*, relationship between growth rates and urea concentrations corresponds to a saturation curve. Similar results were also observed for the red algae *Neogardhiella bailey* (Harvey *ex* Kützing) Wynne & Taylor and *Gracilaria foliifera* (Forsskal) Børgesen, with half saturation constants (K_s) values ranging from 0.2 to 0.4 μM of different nitrogen sources, and maximum values of growth rates at nitrogen concentrations up to 1.0 μM (DeBoer *et al.* 1978). However, higher values were observed to young specimens of the brown algae *Macrocystis pyrifera* (L.) Agardh (from 6 to 15 μM) and the green algae *Cladophora* aff. *albida* (Hudson) Kützing (30 μM) (Gordon *et al.* 1981). In the present study, optimum concentration was 5 μM N-nitrate for DG morph of *H. musciformis*. Similar results were observed to *Laminaria saccharina* (L.) J.V. Lamour., which growth rate reached the maximum value at nitrogen concentrations of 5 and 10 μM N-nitrate (Wheeler & Weidner 1983). With addition of urea, the optimum concentration was 10 μM for BR-1 and DG morphs. Urea is considered a good nitrogen source for many seaweed species, and, generally its assimilation follows saturation kinetics (Thomas & Harrison 1985).

In conclusion, responses of the four colour morphs showed intraspecific variations in relation to nitrogen sources and concentrations. Moreover, colour morphs of *H. musciformis* could be used as bioindicators of nitrogen pollution in the marine environment by their low tolerance to ammonium.

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Literature cited

- Andria, J.R., Vergara, J.J. & Perez-Llorens, J.L.** 1999. Biochemical responses and photosynthetic performance of *Gracilaria* sp. (Rhodophyta) from Cádiz, Spain, cultured under different inorganic carbon and nitrogen levels. *European Journal of Phycology* 34: 497-504.
- Berchez, F.A.S., Pereira, R.T.L. & Kamiya, N.F.** 1993. Culture of *Hypnea musciformis* (Rhodophyta - Gigartinales) on artificial substrates attached to linear ropes. *Hydrobiologia* 260/261: 415-420.
- Bird, K.T., Habig, C. & Debusk, T.** 1982. Nitrogen allocation and storage patterns in *Gracilaria tikvahiae* (Rhodophyta). *Journal of Phycology* 18: 344-348.
- Braga, A.C. & Yoneshigue-Valentin, Y.** 1994. Growth of *Laminaria abyssalis* (Phaeophyta) at different nitrate concentrations. *Phycologia* 33: 271-274.
- Braga, A.C. & Yoneshigue-Valentin, Y.** 1996. Nitrogen and phosphorus uptake by the Brazilian kelp *Laminaria abyssalis* (Phaeophyta) in culture. *Hydrobiologia* 326/327: 445-450.
- Chow, F., Capociana, F.V., Faria, R.B.M. & Oliveira, M.C.** 2007. Characterization of nitrate reductase activity in vitro in *Gracilaria caudata* J. Agardh (Rhodophyta, Gracilariales). *Revista Brasileira de Botânica* 30: 123-129.
- Collén, P.N., Camitz, A., Hancock, R.D., Viola, R. & Pedersén, M.** 2004. Effect of nutrient deprivation and resupply on metabolites and enzymes related to carbon allocation in *Gracilaria tenuistipitata* (Rhodophyta). *Journal of Phycology* 40: 305-314.
- Costa, V.L.** 2005. Diversidade intraespecífica em gametófitos de *Gracilaria birdiae* (Gracilariales, Rhodophyta): efeitos fisiológicos da concentração de nitrogênio no meio de cultura. Tese de Doutorado, Universidade de São Paulo, São Paulo.
- Critchley, A.T. & Ohno, M.** 1998. Seaweed resources of the world. Japan International Cooperation Agency, Yokosuka.
- Deboer, J.A., Guigli, H.J., Israel, T.L. & D'Elia, C.F.** 1978. Nutritional studies of two red algae. I. Growth rate as a function of nitrogen source and concentration. *Journal of Phycology* 14: 261-266.
- D'Elia, C.F. & Deboer, J.A.** 1978. Nutritional studies of two red algae. II. Kinetics of ammonium and nitrate uptake. *Journal of Phycology* 14: 226-272.
- Donato, R.** 2005. Diversidade intraespecífica em linhagens tetrasporofíticas de *Gracilaria birdiae* (Gracilariales, Rhodophyta): crescimento, caracterização pigmentar, fotossíntese e assimilação de nitrato. Tese de doutorado, Universidade de São Paulo, São Paulo.
- Edwards, P.** 1970. Illustrated guide to the seaweeds and seagrasses in the vicinity of Porto Aransas, Texas. *Contributions to Marine Science, University of Texas at Austin* 15: 1-228.
- Fujita, R.M.** 1985. The role of nitrogen status in regulating transient ammonium uptake and nitrogen storage by macroalgae. *Journal of Experimental Marine Biology and Ecology* 92: 283-301.
- García-Sánchez, M.J., Fernández, J.A. & Niell, F.X.** 1993. Biochemical and physiological responses of *Gracilaria tenuistipitata* under two different nitrogen treatments. *Physiological Plantarum* 88: 631-637.
- Gerung, G.S. & Ohno, M.** 1997. Growth rates of *Eucheuma denticulatum* (Burman) Collins et Harvey and *Kappaphycus striatum* (Schmitz) Doty under different conditions in warm waters of Southern Japan. *Journal of Applied Phycology* 9: 413-415.
- Gordon, D.M., Birch, P.B. & McComb, A.J.** 1981. Effects of inorganic phosphorus and nitrogen on the growth of an estuarine *Cladophora* in culture. *Botanica Marina* 24: 93-106.
- Granbom, M., Chow, F., Lopes, P.F., Oliveira, M.C., Colepicolo, P., Paula, E.J. & Pedersén, M.** 2004. Characterisation of nitrate reductase in the marine macroalgae *Kappaphycus alvarezzi* (Rhodophyta). *Aquatic Botany* 78: 295-305.
- Haines, K.C.** 1976. Growth of the carrageenan-producing tropical red seaweed *Hypnea musciformis* in surface water, 870m deep water, effluent from a clam mariculture system, and in deep water enriched with artificial fertilizers or domestic sewage. In: F. Persson & E. Jaspers (eds.). *Proceedings of 10th European Symposium*

- on Marine Biology, v. I: Mariculture. Universal Press, Wetteren, pp. 207-220.
- Haines, K.C. & Wheeler, P.A.** 1978. Ammonium and nitrate uptake by the marine macrophytes *Hypnea musciformis* (Rhodophyta) and *Macrocystis pyrifera* (Phaeophyta). *Journal of Phycology* 14: 319-324.
- Hanisak, M.D.** 1983. The nitrogen relationships of marine macroalgae. In: E.J. Carpenter & D.G. Capone (eds.) *Nitrogen in the Marine Environment*. Academic Press, New York, pp. 699-730.
- Hanisak, M.D.** 1990. The use of *Gracilaria tikvahiae* (Gracilariales, Rhodophyta) as a model system to understand the nitrogen nutrition of cultured seaweeds. *Hydrobiologia* 204/205: 79-87.
- Hurd, C.L., Berges, J.A., Osborne, J. & Harrison, P.J.** 1995. An *in vitro* nitrate reductase assay for marine macroalgae: optimization and characterization of the enzyme for *Fucus gardneri* (Phaeophyta). *Journal of Phycology* 31: 835-843.
- Hwang, S.-P.L., Williams, S.L. & Brinkhuis, B.H.** 1987. Changes in internal dissolved nitrogen pools as related to nitrate uptake an assimilation in *Gracilaria tikvahiae* McLachlan (Rhodophyta). *Botanica Marina* 30: 11-19.
- Iwasaki, H.** 1961. The life cycle of *Porphyra tenera* in vitro. *Biological Bulletin* 121: 173-187.
- Lobban, C.S. & Harrison, P.J.** 1994. *Seaweed Ecology and Physiology*. Cambridge University Press, Cambridge.
- Lopes, P.F., Oliveira, M.C. & Colepicolo, P.** 2002. Characterization and daily variation of nitrate reductase in *Gracilaria tenuistipitata* (Rhodophyta). *Biochemical and Biophysical Research* 295: 50-54.
- Martínez, B. & Rico, J.M.** 2004. Inorganic nitrogen and phosphorus uptake kinetics in *Palmaria palmata* (Rhodophyta). *Journal of Phycology* 40: 642-650.
- Martins, A.P., Yokoya, N.S., Carvalho, M.A.M. & Plastino, E.M.** 2008. Effects of kinetin and nitrogen on growth rates, pigment and protein contents of wild and phycoerythrin-deficient morphs of *Hypnea musciformis* (Rhodophyta). *Journal of Applied Phycology* (in press). DOI 10.1007/s10811-007-9289-x.
- Masuda, J., Yamagishi, Y., Chiang, Y.-M., Lewmanomont, K. & Xi, B.** 1997. Overview of *Hypnea* (Rhodophyta, Hypneacea). In: I.A. Abbott (ed). *Taxonomy of economic seaweeds*. California Sea Grant College System, La Jolla, v. 6, pp. 127-133.
- Mohsen, A.F., Khaleafa, A.F., Hashem, M.A. & Metwalli, A.** 1974. Effect of different nitrogen sources on growth, reproduction, amino acid, fat and sugar contents in *Ulva fasciata* Delile. *Botanica Marina* 17: 218-222.
- Naldi, M. & Wheeler, P.A.** 2002. ¹⁵N measurements of ammonium and nitrate uptake by *Ulva fenestrata* (chlorophyta) and *Gracilaria pacifica* (Rhodophyta): comparison of net nutrient disappearance, release of ammonium and nitrate, and ¹⁵N accumulation in algal tissue. *Journal of Phycology* 38: 135-144.
- Nasr, A.H., Bekheet, I.A. & Ibrahim, R.K.** 1968. The effect of different nitrogen and carbon sources on amino acid synthesis in *Ulva*, *Dictyota*, and *Pterocladia*. *Hydrobiologia* 31: 7-16.
- Navarro-Angulo, L. & Robledo, D.** 1999. Effects of nitrogen source, N:P ratio and N-pulse concentration and frequency on the growth of *Gracilaria cornea* (Gracilariales, Rhodophyta) in culture. *Hydrobiologia* 398/399: 315-320.
- Paula, E.J., Pereira, R.T.L. & Ohno, M.** 1999. Morph selection in *Kappaphycus alvarezii* var. *alvarezii* (Solieriaceae, Rhodophyta) using tetraspore progeny. *Journal of Applied Phycology* 11: 111-121.
- Pedersen, A., Kraemer, G. & Yarish, C.** 2004. The effects of temperature and nutrient concentrations on nitrate and phosphate uptake in different species of *Porphyra* from Long Island Sound (USA). *Journal of Experimental Marine Biology and Ecology* 312: 235-252.
- Phillips, J.C. & Hurd, C.L.** 2004. Kinetics of nitrate, ammonium, and urea uptake by four intertidal seaweeds from New Zealand. *Journal of Phycology* 40: 534-545.
- Plastino, E.M.** 2003. Cultivo en laboratorio y selección de cepas en algas rojas (Rhodophyta). In: A. Mansilla, C. Werlinger & N. Navarro (eds.). *Memorias de Curso Internacional de Posgrado y Especialización de Macroalgas em Ambientes Subantárticos*. Ediciones Universidad de Magallanes, Punta Arenas, pp. 53-58.
- Prince, J.S.** 1974. Nutrient assimilation and growth of some seaweeds in mixtures of seawater and secondary sewage treatment effluents. *Aquaculture* 4: 69-79.

- Rossa, M.M.** 1999. A luz como fator de regulação da atividade enzimática da nitrate redutase (NR) e superóxido dismutase (SOD) em *Gracilariopsis tenuifrons* (Rhodophyta). Dissertação de Mestrado, Universidade de São Paulo, São Paulo.
- Taylor, M.W. & Rees, T.V.** 1999. Kinetics of ammonium assimilation in two seaweeds, *Enteromorpha* sp. (Chlorophyceae) and *Osmundaria colensoi* (Rhodophyceae). *Journal of Phycology* 35: 740-746.
- Thomas, T.E. & Harrison, P.J.** 1985. Effects of nitrogen supply on nitrogen uptake, accumulation and assimilation in *Porphyra perforata* (Rhodophyta). *Marine Biology* 85: 269-278.
- Turpin, D.H.** 1991. Effects of inorganic N availability on algal photosynthesis and carbon metabolism. *Journal of Phycology* 27: 14-20.
- Ursi, S. & Plastino, E.M.** 2001. Crescimento *in vitro* de linhagens de coloração vermelha e verde clara de *Gracilaria* sp. (Gracilariales, Rhodophyta) em dois meios de cultura: análises de diferentes estádios reprodutivos. *Revista Brasileira de Botânica* 24: 587-594.
- Waite, T.D. & Mitchell, R.** 1972. The effect of nutrient fertilization on the benthic alga *Ulva lactuca*. *Botanica Marina* 15: 151-156.
- Wheeler, W.N. & Weidner, M.** 1983. Effects of external inorganic nitrogen concentration on metabolism, growth and activities of key carbon and nitrogen assimilating enzymes of *Laminaria saccharina* (Phaeophyceae) in culture. *Journal of Phycology* 19: 92-96.
- Yan, X.-H., Fujita, Y. & Aruga, Y.** 2000. Induction and characterization of pigmentation mutants in *Porphyra yezoensis* (Bangiales, Rhodophyta). *Journal of Applied Phycology* 12: 69-81.
- Yokoya, N.S.** 2000. Apical callus formation and plant regeneration controlled by plant growth regulators on axenic culture of the red alga *Gracilariopsis tenuifrons* (Gracilariales, Rhodophyta). *Phycological Research* 48: 133-142.
- Yokoya, N.S., Plastino, E.M. & Artel, R.** 2003. Physiological responses and pigment characterization of two colour morphs of the carrageenophyte *Hypnea musciformis* (Rhodophyta). *In*: A.R.O. Chapman, R.J. Anderson, V.J. Vreeland & I.R. Davison (eds). *Proceedings of the XVIIth International Seaweed Symposium*. Oxford University Press, New York, pp. 425-433.