J Appl Phycol (2014) 26:849–858
DOI 10.1007/s10811-013-0184-3

Intraspecific variation in *Gracilaria caudata* (Gracilariales, Rhodophyta): growth, pigment content, and photosynthesis

F. O. Araújo · S. Ursi · E. M. Plastino

Received: 26 May 2013 / Revised and accepted: 10 October 2013 / Published online: 27 October 2013

Abstract In Brazil, one of the probable reasons for failure in attempts at macroalgal mariculture is the lack of previous studies under controlled conditions. *Gracilaria caudata* is an important marine red alga which is locally exploited for the production of agar. In this study the aim was to compare in vitro growth rates, pigment content, and photosynthesis in gametophytes and tetrasporophytes of *G. caudata* from two distinct geographical areas located 2,500 km apart on the Brazilian coast, one in a warmer area closer to the equator (northeastern population), and the other in a colder area closer to the Tropic of Capricorn (southeastern population). Additionally, the artificial ultraviolet B (UVB) radiation effects on strains were evaluated. Under UVB, deleterious effects were observed in all strains. Although the strains from the southeastern population had higher growth rates than those from the northeastern under control condition, the opposite was observed under UVB condition. Under controlled conditions and regardless of the population, growth rates, net photosynthesis, P_{max}, I_k, and pigment contents were higher in tetrasporophytes than in gametophytes. Consequently, when determining the real potential of a certain phase in cultivation, the tetrasporophyte appears to be the more promising for future experiments along the Brazilian coast. Furthermore, although the growth rate of southeastern strains under control condition was higher, their higher sensitivity to UVB radiation emphasizes the importance of careful selection of the most suitable sites prior to experimental cultivation. The differences in performance between the southeastern and northeastern strains provide support for the hypothesis of their ecotypic differentiation.

F. O. Araújo · S. Ursi · E. M. Plastino (✉)
Departamento de Botânica, Instituto de Biociências, Universidade de São Paulo, Rua do Matão 277, Cidade Universitária
05508-090 São Paulo, SP, Brasil
e-mail: emplasti@usp.br

Keywords Gametophytes · Tetrasporophytes · *Gracilaria* · Growth rates · Photosynthesis · Pigments

Introduction

Species of *Gracilaria* Greville contribute to around 80 % of global agar production, with cultivation on a commercial scale reaching prominence mainly in Chile and Indonesia (Bixler and Porse 2011). Consequently, the genus has been the subject of many studies worldwide (Oliveira and Plastino 1994).

Although various strains with desirable characteristics for mariculture have become valuable tools for inducing increased seaweed productivity (Dawes 1992, 1995; Santelices 2001; Hayashi et al. 2010) this is not the case with *Gracilaria* spp. where no intensive efforts toward strain selection have been undertaken (Levy and Friedlander 1990). Whereas a green strain of *Gracilaria tikvahiae* McLachlan has been used in small-scale commercialization for human consumption (Patwary and van der Meer 1992), the performance of some strains of *Gracilaria birdiae* Plastino and E.C. Oliveira and *Gracilaria cornea* J. Agardh have only been studied under laboratory conditions (Costa and Plastino 2001; Ursi and Plastino 2001; Ursi et al. 2003; Plastino et al. 2004, Ferreira et al. 2006).

Gracilaria has a *Polysiphonia*-type life history with isomorphic gametophytic and tetrasporophytic generations (Oliveira and Plastino 1994). Although isomorphic, and depending on environmental conditions, there is evidence that these phases can respond differently (Destombe et al. 1993). Furthermore, the predominance of tetrasporophytes in *Gracilaria* populations seems to indicate that there is something in this genus that makes the diploid phase fitter (Kain and Destombe 1995). In the laboratory these two life-history phases can be evaluated under controlled conditions,

thereby possibly leading to a better interpretation of their performance.

Over past decades several attempts at macroalgal mariculture along the Brazilian coast have failed, partly through the absence of previous studies under controlled conditions (Costa and Plastino 2011). At present, *Gracilaria caudata* J. Agardh is one of the economically important species that is being exploited along the northeastern coast of Brazil for agar production (Oliveira and Miranda 1998). The species has also proved to be efficient in experimental bioremediation of nutrients from shrimp-farming wastewater (Marinho-Soriano et al. 2009).

Apart from its wide distribution along the Brazilian coast, *G. caudata* is also found in the Caribbean, thereby indicating its occurrence at different latitudes exposed to distinct variations in light, temperature, and nutrient conditions. Common on bedrock, it occurs mostly in protected bays and turbid waters extending from the mesolittoral to the infralittoral fringe (Plastino and Oliveira 1997). The species has a typical *Polysiphonia*-type life history (as *Gracilaria* sp., Oliveira and Plastino 1984) and, under laboratory conditions, tolerates a wide range of salinity (10–60 ups) and temperature (18–30 °C; as *Gracilaria* sp., Yokoya and Oliveira 1992a, b).

In order to study phenotypic plasticity promoted by acclimation and adaptation processes, with the possibility of selecting strains for attempts of mariculture, specimens of *G. caudata* from two distinct geographical areas along the Brazilian coast, both 2,500 km apart, one a warmer area closer to the equator and the other colder closer to the Tropic of Capricorn, were isolated in our laboratory. It was predicted that, as strains from the southeastern and northeastern populations would present variation in growth and photosynthesis rates under laboratory cultivation, these would be physiological ecotypes. Moreover, a further prediction was that tetrasporophytes of *G. caudata* would be more suitable for future mariculture than gametophytes. In this work, a comparison was made of growth, pigment content, and photosynthesis of gametophytes and tetrasporophytes strains from both populations under laboratory-culture conditions. In addition, the effects of artificial ultraviolet B radiation (UVBR) on strains were evaluated. It is also expected that this study will provide information as to the best *G. caudata* candidate for future mariculture in Brazil.

Materials and methods

Strains of *Gracilaria caudata* were obtained from in vitro unialgal non-axenic cultures established as described by Plastino and Oliveira (1990). Female gametophytes (GSP) were collected in southeastern Brazil (Dura Beach, 23°30′ 2.58″S 45°10′31.58″O, Ubatuba, São Paulo State) and tetrasporophytes (TSP) were obtained from cystocarpic plants collected in this population (southeastern strains). Tetrasporophytes (TCE) were collected in northeastern Brazil (Meirelles Beach, 3°43′36.50″S 38°29′54.91″O, Fortaleza, Ceará State), and female gametophytes (GCE) derived from tetraspores released by tetrasporophytes from this population (northeastern strains). So, in all, four strains were used for the experiments: female gametophytes (GCE and GSP) and tetrasporophytes (TCE and TSP). Strains were maintained in the Gracilariaceae germplasm bank of the University of São Paulo (Costa et al. 2012). Voucher specimens were deposited in the herbarium of University of São Paulo (GCE, SPF 57175; TCE, SPF 57173; GSP, SPF 57171; and TSP, SPF 57176).

General culture conditions

Strains were maintained in sterile seawater (32 psu salinity), enriched with 25 % von Stosch solution (Ursi and Plastino 2001). The algae were kept in a controlled environment at 25 ±1 °C with a photoperiod of 14 h light and 10 h dark. The photosynthetic active radiation (PAR) was 70 μmol photons m^{-2} s^{-1} provided by 40 W daylight fluorescent tubes and measured by a quantameter (Li-COR model L1-185). Cultures were aerated for 30 min h^{-1}, and the medium was renewed weekly.

Experimental irradiance conditions

Two treatments were performed (control and UVBR). Control treatment was similar to general culture conditions, whereas in the other treatment, 0.08 W m^{-2} of UVBR exposure was provided for 3 h day^{-1} in the middle of the light period. UVBR was supplied by UV-B Broad Band lamps (20 W/12 RS, Phillips TL) and measured using a radiometer (Vilber-Lourmat VLX-3 W no. 2218). Experiments were conducted for a period of 28 days. Three replicates per treatment were made for each strain (GCE, TCE, GSP, and TSP).

Growth rates

One week before the beginning of the experiment (pre-treatment), 24 apical fragments of each strain (GCE, TCE, GSP, and TSP), approximately 2 cm in length, were cultivated in six polypropylene flasks containing 400 mL of enriched seawater (about 40 mg of algae $flask^{-1}$) and covered with plastic film of polyvinyl chloride (PVC, Vitaspenser Goodyear) in the absence of UVBR. After that, these strains were cultivated in triplicate in each experimental condition for growth rate determination (control and UVBR). Growth was assessed weekly by measures of mass, whereas the morphology was recorded at the beginning and after 4 weeks of the experiment. The growth rates were estimated by means of the following formula: $GR=[(M_f/M_i)^{1/t}-1]\times 100$ %, where

M_f is the final mass, M_i is the initial mass, and t is the time (Lignell and Pedersén 1989). Analyses of variance (ANOVAs) were performed, followed by Newman–Keuls a posteriori tests: two-factor (strain and site as independent variables); and three-factor (strain, site, and treatment as independent variables). Confidence interval utilized was at 95 % ($p<0.05$).

Pigment analyses

Phycobiliproteins, chlorophyll *a*, and carotenoids were quantified in all strains cultivated in the control and UVBR conditions for 4 weeks. ANOVA were performed on all data, followed by a Newman–Keuls a posteriori test when necessary [independent variables (three-factor): strain, site, and treatment]. Confidence interval utilized was at 95 % ($p<0.05$).

Phycobiliproteins and chlorophyll a

Pigment extractions were carried out at 4 °C, according to Kursar et al. (1983) with modifications (Plastino and Guimarães 2001). Briefly, 300 mg of apical segments were disrupted by grinding with liquid nitrogen and 50 mM L^{-1} phosphate buffer pH 5.5. Crude extracts were centrifuged at 44,000×*g* for 20 min. The supernatant containing the phycobiliproteins was removed and kept in sealed vials at 4 °C until a reading was taken using a spectrophotometer (HP 8452A). Chlorophyll *a* (Chl *a*) was extracted after dissolving the pellet in 90 % acetone and then centrifuged at 12,000×g for 15 min. Pigment concentration was calculated according to Kursar et al. (1983) for phycobiliproteins [phycoerythrin (PE), phycocyanin (PC), and allophycocyanin (APC)] and for Chl *a*, according to Jeffrey and Humphrey (1975). All pigment extractions were performed in triplicate.

Total carotenoids

Pigment extractions were carried out from samples of approximately 30 mg fresh weight for each repetition (total: three repetitions). Briefly, 2 mL of dimethylformamide (99.8 %) was added to each sample. The samples were kept at 4 °C in the dark for 12 h. Crude extracts were centrifuged at 19,000×*g* for 20 min. The supernatant containing carotenoids was transferred to test tubes which have remained sealed in the dark at 4 °C until the reading on the spectrophotometer (Wellburn 1994). We used the equation described by Wellburn (1994) to determine the concentration of total carotenoids.

Photosynthesis rates

All strains previously cultivated in the control and UVBR conditions were available (three replicates of 300 mg treatment^{-1}). The light and dark bottle method (Littler and Arnold 1985; Thomas 1988) was used to measure net photosynthesis and respiration rates as dissolved oxygen evolution. The incubation bottles had 170 mL. An oximeter (YSI model 58 portable dissolved oxygen meter) with automatic electrode (YSI 5905) was used to measure dissolved oxygen concentrations every 5 min for 1 h in each replicate (three replicates). Data used to construct photosynthesis versus irradiance curves ($P\times I$) were taken at seven levels of irradiance: 10, 50, 100, 200, 400, 800, and 1,600 μmol photons m^{-2} s^{-1} (white light, fluorescent "daylight" Osram 40 W). Respiratory rates were measured in the dark. The incubations were always carried out between 12:00 and 13:00 h (after 3–4 h of exposure to light in the culture chamber) to avoid possible effects of circadian rhythms. Temperature incubations were kept at 25±1 °C. The net photosynthesis rates obtained in PAR and PAR+UVBR for each strain (TSP, GSP, TCE, and GCE) were statistically compared by two types of ANOVA (two-factor), followed by a Newman–Keuls a posteriori test when necessary. In one of them, the independent variables were: intensity of irradiance and treatment (PAR and PAR+UVBR), and in other, the variables were intensity of irradiance and strain. In the latter, the data obtained in control and UVBR conditions were analyzed separately. Confidence interval utilized was at 95 % ($p<0.05$). Respiratory rates were also statistically compared by ANOVA (two-factor, independent variables: reproductive phase and site).

Data used to construct photosynthesis versus irradiance ($P\times I$) curves were the values of dissolved oxygen ($n=3$ strain^{-1}). The curves were fitted using Eq. 2 of Henley (1993): $P=P_{max}\{\alpha I/[P_{max}{}^2+(\alpha I)^2]^{1/2}\}+R$, where P=net photosynthesis, I=irradiance, and R=respiration in the dark. The curve parameters were calculated according to Platt et al. (1980): F_{max} (maximum photosynthesis), α (photosynthetic efficiency), I_k (light saturation parameter, calculated from F_{max}/α), and I_c (compensation irradiance). Each of the parameters obtained from the $P\times I$ curves (P_{max}, α, I_k, I_C, and P_{max} R/R) were compared by ANOVA (two-factor, independent variables: strain and treatment).

Results

Growth rates

Under controlled conditions, *Gracilaria caudata* was healthy throughout the experiment. However, apices exposed to UVBR treatment became paler, and some of them showed curling of tips. This morphological alteration was more evident after 21 days. These strains showed lower growth rates than those cultivated under control condition ($F=189.384$, $p<0.05$; Fig. 1). The GR of tetrasporophytes,

regardless of the treatment or population (north or south), were higher when compared to GR of gametophytes (F = 277.84, p <0.05). In control condition, southeastern strains showed higher growth rates than northeastern strains (F = 364.48, p <0.05), whereas under UVBR, the latter growth was higher than the southeastern strains (F =89.436, p <0.05).

Pigment content

Since all pigments were observed in all strains, albeit with different concentrations, no qualitative differences were evident. The phycobiliprotein extracts of all strains had absorption peaks at 496, 564, and 618 nm, whereas the Chl *a* extracts had absorption peaks at 430 and 664 nm.

Phycoerythrin

The strains cultivated under control condition had higher concentrations of PE than strains exposed to UVBR treatment (F =1341.70, p <0.05; Fig. 2a). In control condition, TCE had higher concentrations of this pigment than GSP (F =2.28, p < 0.05), whereas GCE and TSP showed intermediate and similar concentrations (F =0.40, p <0.05). No differences in concentration of PE were found among strains when exposed to UVBR treatment (F =44.70, p >0.05).

Phycocyanin

The strains cultivated under control condition had higher concentrations of PC than strains exposed to UVBR treatment (F =18.88, p <0.05; Fig. 2b). In both treatments, TCE had higher concentrations of this pigment when compared to the other strains. In control condition, tetrasporophyte strains (TCE and TSP) had higher concentrations when compared to gametophytes, and GCE had higher concentrations than GSP (F =1.15, p <0.05). Under UVBR treatment, TCE had higher concentrations of PC than GSP, whereas GCE and TSP showed intermediate and similar concentrations (F =2.06, p <

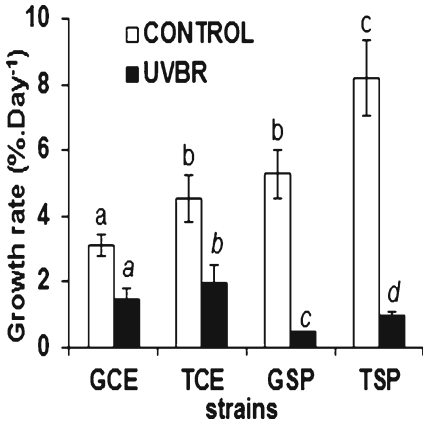

Fig. 1 Growth rates of tetrasporophytes and gamethophytes of *Gracilaria caudata* from distinct sites of Brazil (CE-Ceará, SP-São Paulo) cultivated for 28 days under control and UVB radiation conditions (UVB=0.08 W m^{-2} for 3 h day^{-1}). Data are mean±SD (n =3). Different letters indicate significant differences according to ANOVA and Newman–Keuls test (P <0.05)

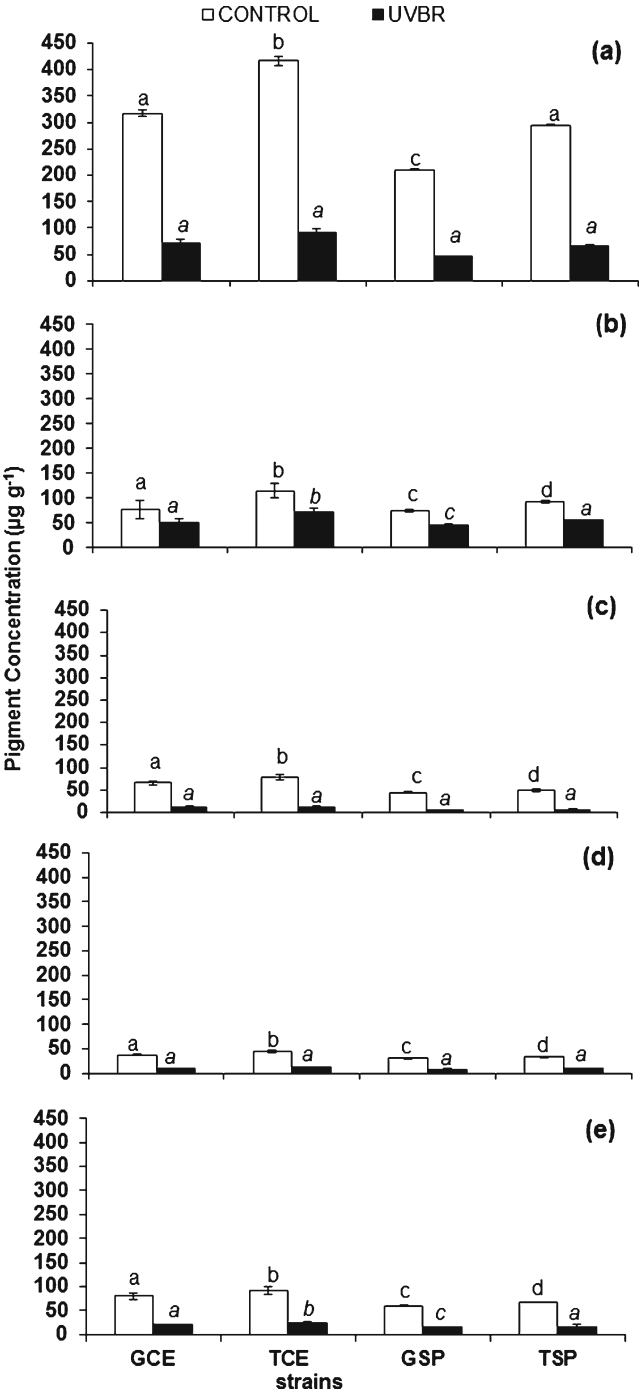

Fig. 2 Pigment concentrations of tetrasporophytes (T) and gametophytes (G) of *Gracilaria caudata* from distinct sites of Brazil (CE-Ceará, SP-São Paulo) cultivated for 28 days under control and UVB radiation conditions (UVB=0.08 W m^{-2} for 3 h day^{-1}). **a** phycoerytrhrin, **b** phycocyanin, **c** allophycocyanin, **d** chlorophyll-a, and **e** carotenoids. Data are mean±SD (n =3). Different letters indicate significant differences according to ANOVA and Newman–Keuls test (P <0.05)

Table 1 Ratios of pigment concentration of tetrasporophytes (T) and gametophytes (G) of *Gracilaria caudata* from distinct sites of Brazil (CE-Ceará, SP-São Paulo) cultivated for 28 days under control and UVB radiation conditions (UVB=0.08 W m^{-2} for 3 h day^{-1})

		PE/PC	PE/APC	PE/Chl-a	PC/APC	PC/Chl-a	APC/Chl-a
GCE	CONTROL	3.61	5.26	9.62	1.46	2.68	1.83
	UVBR	1.28	6.91	8.03	5.37	6.24	1.16
TCE	CONTROL	4.08	4.81	8.45	1.18	2.07	1.75
	UVBR	1.16	6.93	6.86	5.96	5.89	0.99
GSP	CONTROL	3.16	6.06	8.79	1.92	2.78	1.45
	UVBR	1.16	8.84	7.00	7.60	6.00	0.79
TSP	CONTROL	2.81	4.72	6.50	1.68	2.48	1.48
	UVBR	0.99	6.34	5.58	6.36	5.59	0.88

PE phycoerytrhrin, *PC* phycocyanin, *APC* allophycocyanin, *Chl-a* chlorophyll-*a*

0.05). PE/PC ratios were lower in UVBR treatment when compared to the control (Table 1).

Allophycocyanin

The strains cultivated under control condition had higher concentrations of APC than strains exposed to UVBR treatment ($F=107.80$, $p<0.05$; Fig. 2c). In control condition, TCE and GCE strains had higher concentrations of this pigment than southeastern strains (TSP and GSP), and tetrasporophyte strains had higher concentrations when compared to gametophytes from the same population ($F=2.16$, $p<0.05$). No differences in concentration of APC were found among strains when exposed to UVBR treatment ($F=2.16$, $p>0.05$). PE/APC and PC/APC ratios were higher in UVBR treatment when compared to the control (Table 1).

Chlorophyll a

The strains cultivated under control condition had higher concentrations of chlorophyll *a* than strains exposed to UVBR treatment ($F=1730.70$, $p<0.05$; Fig. 2d). In control condition, TCE and GCE strains had higher concentrations of this pigment than southeastern strains (TSP and GSP), and tetrasporophyte strains had higher concentrations when compared to gametophytes from the same population ($F=1.68$, $p<0.05$). No differences in concentration of chlorophyll *a* were found among strains when exposed to UVBR treatment ($F=1.10$, $p>0.05$).

Total carotenoids

The strains cultivated under control condition had higher concentrations of carotenoids than strains exposed to UVBR treatment ($F=3{,}103.30$, $p<0.05$; Fig. 2e). In both treatments, TCE and GCE strains had higher concentrations of these pigments than strains from southeastern population (TSP and GSP), and tetrasporophyte strains had higher concentrations when compared to gametophytes from the same population ($F=2.65$, $p<0.05$).

Photosynthesis rates

In all incubations, P_{max} was reached below 1,000 μmol photons m^{-2} s^{-1}, and no photoinhibition was observed up to the maximum light level of 1,600 μmol photons m^{-2} s^{-1}. All four strains of *G. caudata* showed a similar pattern of photosynthetic rates when subjected to increasing irradiance,

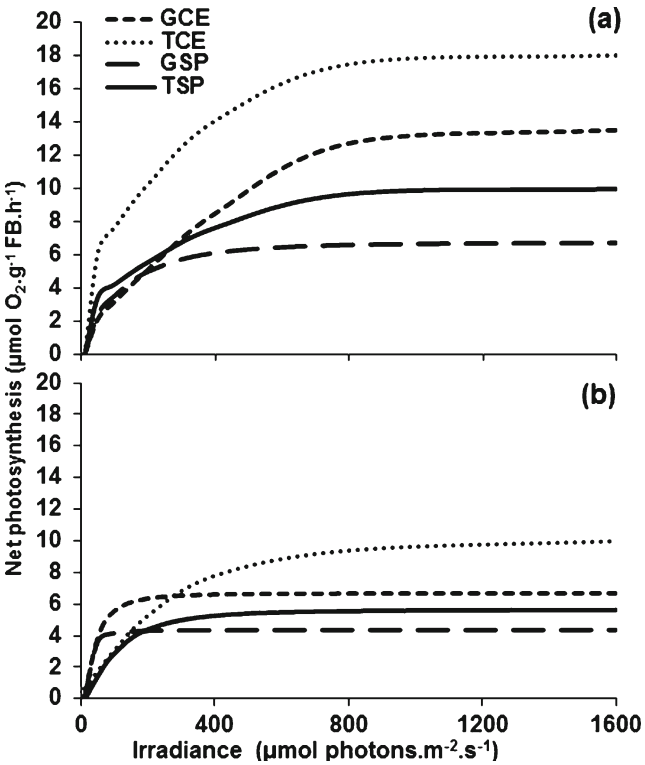

Fig. 3 Photosynthesis versus irradiance curves of tetrasporophytes (*T*) and gamethophytes (*G*) of *Gracilaria caudata* from distinct sites of Brazil (CE-Ceará, SP-São Paulo) cultivated for 28 days under control (**a**) and UVB radiation conditions (**b**) (UVB=0.08 W m^{-2} for 3 h day^{-1})

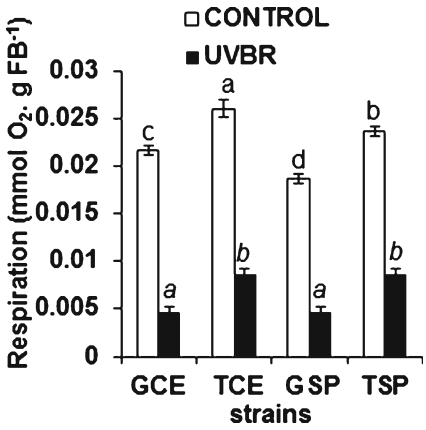

Fig. 4 Respiration rates of tetrasporophytes (*T*) and gametophytes (*G*) of *Gracilaria caudata* from distinct sites of Brazil (CE-Ceará, SP-São Paulo) cultivated for 28 days under control and UVB radiation conditions (UVB=0.08 W m^{-2} for 3 h day^{-1}). Data are mean±SD ($n=3$). Different letters indicate significant differences according to ANOVA and Newman–Keuls test ($P<0.05$)

and the same mathematical model to fit the $P \times I$ curves could be used (Fig. 3). However, some quantitative differences were found among the net photosynthesis rates, net respiration rates, and $P \times I$ curve parameters of strains and treatments.

The strains cultivated under control condition had higher photosynthesis rates when compared to strains exposed to UVBR treatment: TCE ($F=103.28$, $p<0.05$), TSP ($F=58.79$, $p<0.05$), GCE ($F=14.89$, $p<0.05$), and GSP ($F=3.11$, $p<0.05$; Fig. 4). In both conditions, TCE had higher photosynthesis rates than GSP, whereas GCE and TSP showed intermediate and similar concentrations (PAR: $F=192.29$, $p<0.05$; PAR+UVBR: $F=113.50$, $p<0.05$).

The strains cultivated under control condition had higher respiration rates when compared to strains exposed to UVBR treatment (Fig. 4). In control condition, all strains showed different rates. The highest rates were observed to TCE, followed by TSP, GCE, and GSP, respectively (reproductive state $F=121.50$, $p<0.05$; locality $F=64.54$, $p<0.05$). Under UVBR treatment, tetrasporophyte strains had similar and higher respiration rates than gametophytes that had similar rates ($F=106.40$, $p<0.05$); there was no difference between southeastern and northeastern strains ($F=48.23$, $p>0.05$).

Considering the $P \times I$ parameters, the highest values of I_k ($F=56.69$, $p<0.05$), P_{max} ($F=32.76$, $p<0.05$), and P_{max}/Re ($F=32.77$, $p<0.05$) were observed in control condition for all strains, while the highest values of I_c were observed in UVBR ($F=36.79$, $p<0.05$). No differences were observed to the other parameters: R ($F=7.34$, $p>0.05$), and α ($F=0.57$, $p>0.05$; Table 1). Regardless of the treatment, no significant differences between strains were observed to Re ($F=3.55$, $p>0.05$), I_c ($F=0.61$, $p>0.05$), and α ($F=0.33$, $p>0.05$). However, I_k ($F=45.79$, $p<0.05$), P_{max}/R ($F=9.36$, $p<0.05$), and P_{max} ($F=10.67$, $p<0.05$) were higher for northeastern strains when compared to southeastern strains. Furthermore, tetrasporophytes had higher values of these three parameters when compared to gametophytes from the same location (Table 2).

Discussion

Growth responses

On comparing strains of the very same *G. caudata* population, regardless of treatment (control or UVBR), tetrasporophytes presented higher growth rates. The highest rates were also recorded for the tetrasporophytes of *Gracilaria tenuistipitata* Chang and Xia when compared to the gametophytes (Barufi et al., 2010). However, in *G. birdiae* (Ursi and Plastino 2001), and *Gracilaria dura* (C. Agardh) J. Agardh (Gupta et al. 2011), female gametophytes presented

Table 2 Photosynthesis properties of tetrasporophytes (T) and gametophytes (G) of *Gracilaria caudata* from distinct sites of Brazil (CE-Ceará, SP-São Paulo) cultivated for 28 days under control and UVB radiation conditions (UVB=0.08 W m^{-2} for 3 h day^{-1})

	GCE		TCE		GSP		TSP	
	Control	UVBR	Control	UVBR	Control	UVBR	Control	UVBR
R	0.95	0.87	0.94	0.95	0.82	0.81	0.92	0.8
P_{max}	11.73±0.02	5.80±0.02	17.98±0.02	9.83±0.02	6.72±0.02	3.98±0.02	9.97±0.03	4.84±0.02
A	0.05±0.00	0.03±0.00	0.05±0.00	0.03±0.00	0.05±0.00	0.04±0.00	0.05±0.00	0.03±0.00
R_d	0.33±0.36	0.32±0.09	0.34±0.49	0.33±0.18	0.34±0.28	0.30±0.03	0.33±0.32	0.32±0.05
I_k	234.6±0.04	207.1±0.02	359.6±0.04	351.1±0.03	140.0±0.04	112.1±0.02	199.4±0.03	179.3±0.03
I_c	6.58±0.00	11.39±0.00	6.78±0.00	11.75±0.00	7.06±0.00	8.42±0.00	6.58±0.00	11.78±0.00
P/R_d	35.65	18.18	53.04	29.88	19.82	13.31	30.31	15.22

Data are mean±SD ($n=3$)

R *R*-value of the Levenberg–Marquadt algorithm to fit $P \times I$ curve, P_{max} light-saturated net photosynthesis rate (mg O_2 g^{-1} fresh biomass h^{-1}), α initial slope at limiting irradiance levels [(mg O_2 g^{-1} fresh biomass h^{-1}) (μmol photons m^{-2} s^{-1})$^{-1}$], R_d respiration rate in darkness (mg O_2 g^{-1} fresh biomass h^{-1}), I_k light saturation parameter (μmol photons m^{-2} s^{-1}), I_c compensation irradiance (μmol photons m^{-2} s^{-1})

higher growth rates than tetrasporophytes. On the other hand, and depending on the culture conditions, Destombe et al. (1993) reported a variety of responses for *Gracilaria gracilis* (Stackhouse) Steentoft, Irvine and Farnham (as *Gracilaria verrucosa*), whereas tetrasporophytes growth rates were high under optimal culture conditions in gametophytes, this was so when culture conditions were less favorable. This apparent inconsistency in phase performance, as reported in the literature, can be attributed to experimental cultivation conditions, such as those related to the quality and quantity of light and nutrient concentrations. Possibly, in nature, small variations in environmental conditions may favor one or another phase of life history, thereby attributing benefits to the maintenance of the species in a given environment. These differences in physiological performance in the isomorphic phases are pointed out as advantageous for the maintenance of this type of life history in nature (Hughes and Otto 1999), whence this diversity must be taken into account when selecting the most suitable sites for the mariculture of species with an isomorphic generation life history.

On comparing the growth performance of *G. caudata* strains from different sites, it was found that under controlled conditions, the southeastern strains presented higher growth rates than the northeastern strains, whereas the opposite was the case when exposed to UVBR treatment. These results highlight intraspecific differences and show the greater sensitivity of strains from the southeastern population to UVBR. Incidentally, this population occurs in a site that receives less UV radiation than the northeastern site (Kirchhoff et al. 2000), the latter being apparently better adapted to this condition. Nonetheless, if there is an increase in UVBR on the coastal regions of South America, as predicted in climate studies (Watanabe et al. 2011), strains that occur in this site could possibly become susceptible.

Pigment contents

The strains of *G. caudata* from the northeastern population presented a larger amount of pigments (ex. phycobiliproteins) when compared to those of the southeastern site, especially under controlled conditions. Apart from using phycobiliproteins as a nitrogen source (García-Sánchez et al. 1993; Talarico and Maranzana 2000), their production is a photoprotective mechanism against increased irradiances (Sinha et al. 1995). Therefore, the larger amount of these pigments in strains of *G. caudata* from the population near the equator could be a form of adaptation, since thereabouts strains are subjected to higher irradiance including UVBR than strains from the southeastern population.

Although on considering strains of the same population and when comparing *G. caudata* tetrasporophytes and gametophytes cultivated under controlled conditions, the former showed the highest concentrations of chlorophyll *a*, carotenoids, PE, PC, and APC; under UVBR treatment, tetrasporophytes possessed only higher carotenoids and PC. The higher pigment content in tetrasporophytes could contribute to higher viability of this phase when compared to the haploid. In natural populations, there is a predominance of *G. caudata* tetrasporophytes in comparison to gametophytes, as occurs in other species of *Gracilaria* (Carneiro et al. 2011). In previous studies of *G. gracilis* (as *G. verrucosa*, Destombe et al. 1989) and *G. domingensis* (Kutzing) Sonder ex Dickie (Guimarães et al. 2003), it has been suggested that the higher tetrasporophyte frequencies in natural populations may be due to the higher viability in this phase. Even in isomorphic species, in which adults are morphologically similar, slight differences in the adult phase or among juveniles may play an important ecological role (Hughes and Otto 1999), whereby this diversity must be taken into account in future sea cultivation. In addition, regardless of the condition (control or UVBR), tetrasporophytes of *G. caudata* presented higher growth rates than gametophytes, an important characteristic that must favor this phase in sea cultivation. However, field experiments are needed to assess whether these data obtained in controlled laboratory conditions would remain in the wild.

Gracilaria caudata strains exposed to UVBR underwent a reduction in pigment content evident not only by the observed bleaching during the first weeks of cultivation but also pigment content assessed after 28 days. This variation in color was also observed for other red algae when exposed to UVB radiation (Poppe et al. 2002; Xu and Gao 2007; Navarro et al. 2010). Other species, such as *Gracilaria edulis* and *Gracilaria lemaneiformis*, also showed reduction in phycobiliprotein concentration when exposed to UVBR (Xu and Gao 2007). Phycobiliproteins, especially PE, located on the periphery of phycobilisomes, are promptly affected by UV radiation (Sinha et al. 1995). These pigments play an important role in the acclimatization mechanism in red algae (Figueroa et al. 1997; Roleda et al. 2004a, b; Barufi et al. 2012). The reduction in pigment content of *G. caudata* strains exposed to UVBR occurred simultaneously to the lower growth rates, highlighting the close relationship between these parameters.

Although the carotenoids constitute an important compound in photoprotective mechanisms (Carnicas et al. 1999; Esteban et al. 2009; Figueroa et al. 2008), there was no increase in this concentration in *G. caudata*, when strains were exposed to UVBR. However, the pigment content was only assessed after 28 days of exposure, without the prior detection of any possible increase in carotenoid content. Moreover, other photoprotective compounds, such as mycosporine-like amino acids also capable of playing an important role, have been reported for *Gracilaria* species (Sinha et al. 2000; Cardozo et al. 2006, Barufi et al. 2012).

Exposure to UVBR promoted an increase in the PE/APC and PC/APC ratio in all the *G. caudata* strains, thereby indicating an increase in the proportion of PE and PC in relation to APC and supporting the hypothesis that PE and PC act as part of a protective mechanism (Sinha et al. 1995).

Photosynthesis and respiration rates

Differences in photosynthesis in the *G. caudata* strains were detected between the two sites and the different phases. As observed, growth rates and pigment content in gametophytes were lower than in tetrasporophytes. The increased P_{max} and I_k in tetrasporophytes imply this phase to be better adapted to higher-light conditions than gametophytes. The lower photosynthetic rates, P_{max} and I_k of southeastern strains suggest a higher sensitivity to light when compared to the northeastern. Based on our results, it may be hypothesized that these populations are ecotypes of *G. caudata*. A similar hyphothesis has already been considered for *G. birdiae*, whose strains from different localities also presented variations in $P \times I$ parameters (Ursi et al. 2003).

As to UVBR exposure, photosynthetic rates in all *G. caudata* strains presented a strong decline when so exposed (TCE 33.3 %, GCE 44.4 %, TSP 50 %, and GSP 60 %). These results are in agreement with the lower growth rates and pigment content observed on strains exposed to UVBR. Some $P \times I$ curve parameters, such as P_{max} and I_k, also had lower values when compared to the control condition. This negative influence of UVBR on photosynthetic rates was also observed for *G. lemaneiformis*, regardless of time of exposure (Gao and Xu 2008; Zheng and Gao 2009).

As detected in photosynthesis, growth rates, and pigment content, there was a decline in *G. caudata* respiration rates when exposed to UVBR treatment, regardless of the site or phase. Our results agree with those obtained for other macroalgae (Häder and Schäfer 1994). The negative influence of UVB treatment was stronger in photosynthesis than in respiration. Aguilera et al. (1999) emphasized that this is to be expected, since mitochondria in macroalgae are numerous, small, and often located in the inner parts of the cells opposite to the thallus surface, whereas the single or few relatively large chloroplasts occupy the area adjacent to the outer cell walls.

In conclusion, *G. caudata* has isomorphic gametophytes and tetrasporophytes which, when considering the parameters analyzed, responded differently. When considering the different strains from the same site and cultivated under identical controlled conditions, the growth rates, net photosynthesis, P_{max}, I_k, and pigment contents in tetrasporophytes were higher than in gametophytes. Thus, tetrasporophytes appear to be the more promising candidates for future experiments along the Brazilian coast, when determining the real potential of a phase in cultivation. Furthermore, the higher growth rate in the southeastern strains under the applied controlled conditions is noteworthy. Nonetheless, their higher sensitivity to UVBR highlights the importance of selecting the most suitable sites to their future experimental cultivation.

Acknowledgments This research was supported by the São Paulo Research Foundation (FAPESP: 2010/50175-3), Brazilian National Council for Scientific and Technological Development (CNPq: 300148/93-3, 134026/2009-8). The authors acknowledge Rosário Petti for assistance on cultivation. We also thank Prof. Dr. Carlos Frederico Martins Menck for providing the radiometer during the course of this work.

References

Aguilera J, Karsten U, Lippert H, Vogele B, Philipp E, Hanelt D, Wiencke C (1999) Effects of solar radiation on growth, photosynthesis and respiration of marine macroalgae from the Arctic. Mar Ecol Prog Ser 191:109–119

Barufi JB, Mata MT, Oliveira MC, Figueroa FL (2012) Nitrate reduces the negative effect of UV radiation on photosynthesis and pigmentation in *Gracilaria tenuistipitata* (Rhodophyta): the photoprotection role of mycosporine-like amino acids. Phycologia 51:636–648

Barufi JB, Oliveira EC, Plastino EM, Oliveira MC (2010) Life history, morphological variability and growth rates of the life phases of *Gracilaria tenuistipitata* (Rhodophyta: Gracilariales) in vitro. Sci Mar 74:297–303

Bixler HJ, Porse H (2011) A decade of change in the seaweed hydrocolloids industry. J Appl Phycol 23:321–335

Cardozo KHM, Carvalho VM, Pinto E, Colepicolo P (2006) Fragmentation of mycosporine-like amino acids by hydrogen/deuterium exchange and electrospray ionization tandem mass spectrometry. Rapid Commun Mass Spectrom 20:253–258

Carneiro MAA, Marinho-Soriano E, Plastino EM (2011) Phenology of an agarophyte *Gracilaria birdiae* Plastino and E.C. Oliveira (Gracilariales, Rhodophyta) in Northeastern Brazil. Rev Bras Farmacogn 21:317–322

Carnicas E, Jiménez C, Niell FX (1999) Effects of changes of irradiance on the pigment composition of *Gracilaria tenuistipitata* var. *liui* Zhang et Xia. J Photochem Photobiol B Biol 50:149–158

Costa E, Plastino EM, Petti R, Oliveira EC, Oliveira MC (2012) The Gracilariaceae Germplasm Bank of the University of São Paulo, Brazil—a DNA barcoding approach. J Appl Phycol 24:1643–1653

Costa VL, Plastino EM (2001) Histórico de vida de espécimens selvagens e variantes cromáticas de *Gracilaria birdiae* (Gracilariales, Rhodophyta). Rev Bras Bot 24:491–500

Costa VL, Plastino EM (2011) Color inheritance and pigment characterization of red (wild-type), greenish-brown, and green strains of *Gracilaria birdiae* (Gracilariales, Rhodophyta). J Appl Phycol 23:599–605

Dawes CJ (1992) Irradiance acclimation of the cultured Philippine seaweeds, *Kappaphycus alvarezii* and *Eucheuma denticulatum*. Bot Mar 35:189–195

Dawes CJ (1995) The effect of nutrient and photon fluence on the photosynthetic responses of red and green pigmented cultivars of *Eucheuma denticulatum*. Bot Mar 38:323–327

Destombe C, Godin J, Nocher M, Richerd S, Valero M (1993) Differences in response between haploid and diploid isomorphic phases of *Gracilaria verrucosa* (Rhodophyta: Gigartinales) exposed to artificial environmental conditions. Hydrobiologia 260/261:131–137

Destombe CM, Valero M, Couvet C (1989) What controls haploid–diploid ratio in the red alga, *Gracilaria verrucosa*? J Evol Biol 2: 317–338

Esteban R, Martínez B, Fernández-Marín B, Becerril JM, García-Plazaola JI (2009) Carotenoid composition in Rhodophyta: insights into xantophyll regulation in *Corallina elongata*. Eur J Phycol 44:221–230

Ferreira LB, Barufi JB, Plastino EM (2006) Growth of red and green strains of the tropical agarophyte *Gracilaria cornea* (Gracilariales, Rhodophyta) in laboratory. Rev Bras Bot 29:185–190

Figueroa FL, Bueno A, Korbee N, Santos R, Mata L, Schuenhoff A (2008) Accumulation of mycosporine-like amino acids in *Asparagopsis armata* grown in tanks with fishpond effluents of gilthead sea bream *Asparus aurata*. J World Aquacult Soc 39: 692–699

Figueroa FL, Salles S, Aguilera J, Jiménez C, Mercado J, Viñegla B, Flores-Moya A, Altamiro M (1997) Effects of solar radiation on photoinhibition and pigmentation in the red alga *Porphyra leucosticta*. Mar Ecol Prog Ser 151:81–90

Gao K, Xu J (2008) Effects of solar UV radiation on diurnal photosynthetic performance and growth of *Gracilaria lemaneiformis* (Rhodophyta). Eur J Phycol 43:297–307

García-Sánchez MJ, Fernández JA, Niell FX (1993) Biochemical and physiological responses of *Gracilaria tenuistipitata* under two different nitrogen treatments. Physiol Plantarum 88:631–637

Guimarães M, Plastino EM, Destombe C (2003) Green mutant frequency in natural populations of *Gracilaria domingensis* (Gracilariales, Rhodophyta) from Brazil. Eur J Phycol 38:165–169

Gupta V, Baghel RS, Kumar M, Kumari P, Mantri VA, Reddy CRK, Jha B (2011) Growth and agarose characteristics of isomorphic gametophyte (male and female) and sporophyte of *Gracilaria dura* and their marker assisted selection. Aquaculture 318:389–396

Häder DP, Schäfer J (1994) Photosynthetic oxygen production in macroalgae and phytoplankton under solar irradiation. J Plant Physiol 144:293–299

Hayashi L, Hurtado AQ, Msuya FE, Bleicher-Lhonneur G, Critchley AT (2010) A review of *Kappaphycus* farming: prospects and constraints. In: Israel A, Einav R, Seckbach J (eds) Seaweeds and their role in globally changing environments. Springer, London, pp 251–283

Henley WJ (1993) Measurement and interpretation of photosynthetic light-response curves in algae in the context of photoinhibition and diel changes. J Phycol 29:729–739

Hughes JS, Otto SP (1999) Ecology and the evolution of biphasic life cycles. Am Nat 154:306–320

Jeffrey SW, Humphrey GF (1975) New spectrophotometric equations for determining chlorophylls a, b, c1 and c2 in higher plants, algae and natural phytoplankton. Biochem Physiol Pflanz 167:191–194

Kain JM, Destombe C (1995) A review of life history, reproduction and phenology of *Gracilaria*. J Appl Phycol 7:269–281

Kirchhoff VWJH, Echer E, Paes Leme N, Silva AA (2000) A variação sazonal da radiação ultravioleta solar biologicamente ativa. Rev Bras Geofis 18:63–74

Kursar TA, van der Meer J, Alberte RS (1983) Light-harvesting system of the red alga *Gracilaria tikvahiae*: I. Biochemical analyses if pigment mutations. Plant Physiol 73:353–360

Levy I, Friedlander M (1990) Strain selection in *Gracilaria* spp. I. Growth, pigment, and carbohydrates of strains of *G. conferta* and *G. verrucosa* (Rhodophyta, Gigartinales). Bot Mar 33:339–345

Lignell A, Pedersén M (1989) Agar composition as a function of morphology and growth rate. Studies on some morphological strains of *Gracilaria secundata* and *Gracilaria verrucosa* (Rhodophyta). Bot Mar 32:219–227

Littler MM, Arnold KE (1985) Electrodes and chemicals. In: Littler MM, Littler DS (eds) Ecological field methods: macroalgae—Handbook of phycological methods. Cambridge University Press, Cambridge, pp 350–375

Marinho-Soriano E, Panucci RA, Carneiro MAA, Pereira DC (2009) Evaluation of *Gracilaria caudata* J. Agardh for bioremediation of nutrients from shrimp farming wastewater. Bioresour Technol 100: 6192–6198

Navarro NP, Mansilla A, Plastino EM (2010) *Iridaea cordata* (Gigartinales, Rhodophyta): responses to artificial UVB radiation. J Appl Phycol 22:385–394

Oliveira EC, Miranda GEC (1998) Aspectos sociais e econômicos da explotação de algas marinhas no Brasil. In: Paula EJ, Cordeiro-Marinho M, Santos DP, Plastino EM, Fujii MT, Yokoya NS (eds) Anais do IV Congresso Latino-americano, II Reunião Ibero-americana, VII Reunião Brasileira de Ficologia. Sociedade Ficológica da America Latina e Caribe & Sociedade Brasileira de Ficologia, Caxambu, MG, pp 149–156

Oliveira EC, Plastino EM (1984) The life-history of *Gracilaria* (Rhodophyta) from Brazil. Jap J Phycol 32:203–208

Oliveira EC, Plastino EM (1994) Gracilariaceae. In: Akatsuka I (ed) Biology of economic algae. SPB Academic, The Hague, pp 185–226

Patwary MU, van der Meer JP (1992) Genetics and breeding of cultivated seaweeds. Korean J Phycol 7:281–318

Plastino EM, Guimarães M (2001) Diversidad intraespecifica. In: Avelã KV, Antezana TJ (eds) Sustentabilidad de la Biodiversidad. Universidad de Concepción, Concepción, pp 19–27

Plastino EM, Oliveira EC (1990) Crossing experiments as an aid to the taxonomic recognition of the agarophytes *Gracilaria*. In: Oliveira EC, Kautsky N (eds) Cultivation of seaweeds in Latin America. Universidade de São Paulo, São Paulo, pp 127–133

Plastino EM, Oliveira EC (1997) *Gracilaria caudata* J. Agardh (Gracilariales, Rhodophyta)—restoring an old name for a common western Atlantic alga. Phycologia 36:225–232

Plastino EM, Ursi S, Fujii MT (2004) Color inheritance, pigment characterization, and growth of a rare light green strain of *Gracilaria birdiae* (Gracilariales, Rhodophyta). Phycol Res 52:45–52

Platt T, Gallegos CL, Harrison WG (1980) Photoinhibition of photosynthesis in natural assemblages of marine phytoplankton. J Mar Res 38:687–701

Poppe F, Hanelt D, Wiencke C (2002) Changes in the ultrastructure, photosynthetic activity and pigments in the Antarctic red algae *Palmaria decipiens* during acclimation to UV radiation. Bot Mar 45:253–261

Roleda MY, Halnet D, Kräbs G, Wiencke C (2004a) Morphology, growth, photosynthesis and pigments in *Laminaria ochroleuca* (Laminariales, Phaeophyta) under ultraviolet radiation. Phycologia 43:603–613

Roleda MY, Van der Poll WH, Halnet D, Wiencke C (2004b) PAR and UVBR effects on photosynthesis, viability, growth and DNA in different life stages of two coexisting Gigartinales: implications for recruitment and zonation pattern. Mar Ecol Prog Ser 281:37–50

Santelices B (2001) Implications of clonal and chimeric-type thallus organization on seaweed farming and harvesting. J Appl Phycol 13:153–160

Sinha RP, Klisch M, Gröniger A, Häder DP (2000) Mycosporine-like amino acids in the marine red alga *Gracilaria cornea*—effects of UV and heat. Environ Exp Bot 43:33–43

Sinha RP, Lebert M, Kumar A, Kumar HD, Häder DP (1995) Spectroscopic and biochemical analyses of UV effects on phycobiliproteins of *Anabaena* sp. and *Nostoc carmium*. Bot Acta 108:87–92

Talarico L, Maranzana G (2000) Light and adaptative responses in red macroalgae: an overview. J Photochem Photobiol B 56:1–11

Thomas WLH (1988) Photosyntesis and respiration of aquatic macro-flora using the light and dark bottle oxygen method and dissolved oxygen analyzer. In: Lobban CS, Chapaman DJ, Kremer BP (eds) Experimental phycology: a laboratory manual. Cambridge University Press, Cambridge, pp 64–77

Ursi S, Pedersén M, Plastino E, Snoeijs P (2003) Intraspecific variation of photosynthesis, respiration and photoprotective carotenoids in *Gracilaria birdiae* (Gracilariales, Rhodophyta). Mar Biol 142: 997–1007

Ursi S, Plastino EM (2001) Crescimento in vitro de linhagens de coloração vermelha e verde clara de *Gracilaria* sp. (Gracilariales, Rhodophyta) em dois meios de cultura: análise de diferentes estádios reprodutivos. Rev Bras Bot 24:587–594

Watanabe S, Sudo K, Nagashima T, Takemura T, Kawase H, Nozawa T (2011) Future projections of surface UV-B in a changing climate. J Geophys Res D 116, D16118. doi:10.1029/2011JD015749

Wellburn AR (1994) The spectral determination of Chlorophylls a and b, as well as total Carotenoids, using various solvents with spectrophotometers of different resolution. J Plant Physiol 144: 307–313

Xu J, Gao K (2007) Growth, pigments, UV-absorbing, compounds and agar yield of economic red seaweed *Gracilaria lemaneiformis* (Rhodophyta) grown at different depths in the coastal waters of South China Sea. J Appl Phycol 20: 681–686

Yokoya NS, Oliveira EC (1992a) Effects of salinity on the growth rate, morphology and water content of some Brazilian red algae of economic importance. Cienc Mar 18:49–64

Yokoya NS, Oliveira EC (1992b) Temperature response of economically important red algae and their potential for mariculture in Brazilian waters. J Appl Phycol 4:339–345

Zheng Y, Gao K (2009) Impacts of solar UV radiation on the photosynthesis, growth, and UV-absorbing compounds in *Gracilaria lemaneiformis* (Rhodophyta) grown at different nitrate concentrations. J Phycol 45:314–323