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Growth, pigments, UV-absorbing compounds and agar yield of the economic red seaweed *Gracilaria lemaneiformis* (Rhodophyta) grown at different depths in the coastal waters of the South China Sea

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Abstract The economic red alga, *Gracilaria lemaneiformis* Bory, was grown at different depths in the coastal waters of the South China Sea, and its growth, pigments, ultra-violet (UV)-absorbing compounds and agar yield were investigated in order to see the impacts of depth change. *Gracilaria lemaneiformis* grew slower at greater depths in March, while the highest relative growth rate (RGR) was found at about 1.0 m depth in April, about 9% higher than that at surface water (0.5 m below the surface). The RGR increased with the increasing daily photosynthetically active radiation (PAR) dose received by the thalli at different depths. The contents of phycoerythrin and chlorophyll *a* increased, while that of UV-absorbing compounds (UVAC, absorption peak at 325 nm) decreased with increased depth. The highest levels of the UVAC in the thalli grown in surface seawater played a protective role against solar UV radiation (280–400 nm). The content of UVAC declined at deeper depths and under indoor low PAR. The agar yield of the thalli increased with the increasing depths, with the highest content found at 3.5 m depth.

Keywords *Gracilaria lemaneiformis* · Growth · Depth · Solar radiation · UV-absorbing substances · Photosynthetic pigments

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Introduction

Macroalgae play an important role in marine primary production in coastal waters. Biomass production of economically important species has potential for remediation of CO_2 and nutrients (Gao and McKinley 1994), and *Gracilaria* is commercially important, accounting for more than 50% of the seaweed used for agar production (Armisen 1995). *Gracilaria lemaneiformis* has been cultivated on large scales in both the southern and the northern parts of China for food and agar production, playing an effective role against coastal eutrophication.

When *G. lemaneiformis* is farmed in the sea, vegetative branches are sandwiched and fixed in ropes which are then hung and floated in surface seawater. As the plants grow with increasing numbers of branches and mass, they are exposed to different quantity and quality of sunlight due to their sinking and expansion to deeper depths. From a photobiological point of view, photosynthetically active radiation (PAR, 400–700 nm) and ultra-violet radiation (UVR) (280–400 nm) attenuate disproportionally with depth, thus different ratios of PAR to UV-A (315–400 nm) or UV-B (280–315 nm) would exert different impacts on *G. lemaneiformis*.

Macroalgae growing at greater depths usually increase their photosynthetic pigments to compensate for the low light availability (Ramus et al. 1976), while those inhabiting shallower water or upper intertidal zone often accumulate UV-absorbing compounds to screen off some extent of UVR (Franklin and Foster 1997). High levels of solar PAR and UVR at noon inhibit photosynthetic performance of macroalgae (Häder et al. 2001). UVR is known to damage DNA (Buma et al. 2001; Zacher et al. 2007) and enzymes (Bischof et al. 2002) of macroalgae. However, positive

effects of UV-A, such as aiding in the recovery process of the inhibited photosynthesis (Flores-Moya et al. 1999), DNA repair (Pakker et al. 2000a,b) and the enhancement of photosynthesis (Viñegla et al. 2006) and growth (Henry and Van Alstyne 2004) have also been suggested.

Previous studies indicate that macroalgae show different ecophysiological characteristics in response to change in growth depth (Ramus et al. 1976; Molloy and Bolton 1996). However, little is known about the relationship between growth and levels of PAR and UVR at different depths, where solar radiation is attenuated to different extents for different wavelengths. With the intention of exploring such a relationship to optimize the cultivation conditions for *G. lemaneiformis*, we investigated its growth rate and contents of photosynthetic pigments, UV-absorbing compounds and agar while growing the thalli at different depths.

Materials and methods

Gracilaria lemaneiformis Bory was collected from its cultivation area at Nanao (116.6°E, 23.3°N), Shantou, China, in March and April 2004. Young plants, about 30 cm long, were selected and maintained in sand-filtered seawater for a night before being returned to the sea and fixed at different depths. The growth experiments were carried out for 12 days in the end of March and 10 days in the middle of April, while the experiments for pigment analyses lasted 45 days from 20 March to 3 May. The daily mean temperature of the surface seawater was 18–19°C in March and 20–22°C in April; salinity of the seawater was constant with a mean value of about 32 psu. During the experiments, the daily dose of solar PAR reaching the sea surface changed from 1.0 to 12.1 MJ m^{-2}. At the site where the plants were hung at different depths, no biomass loss due to grazing was observed.

Measurement of growth

The study area is in a bay with low wave agitation. Thalli of about 10 g (fresh wt) were fixed in nylon ropes and placed at 0.5, 1.0, 1.5, 2.5 and 3.5 m depth by tying the ropes to a floating system supported by float-balls. Triplicate cultures were carried out at each depth, and each culture had 3–4 individuals. The thalli were wrapped in a net (mesh 0.4 cm) and examined by comparing their morphological features at regular intervals; there were no visible losses of the biomass of each individual. Biomass changes were monitored every 3 days for 12 days from 20 March to 2 April and every 2 days for 10 days from 11 to 21 April 2004, respectively. Fresh weight of the thalli was measured after lightly blotting with tissue paper. The relative growth rate (RGR, % day^{-1}) was estimated as follows: $RGR=100\times(lnN_t-lnN_0)/t$, where N_0 is the initial fresh weight and N_t is the fresh weight after t days.

Determination of UV-absorbing compounds and photosynthetic pigments

Concentrations of UV-absorbing compounds (UVAC, absorption peaked at 325 nm) were estimated for the thalli grown at different depths in the sea as well as for those grown under indoor conditions. About 200 mg (fresh wt) thallus was extracted in 20 mL absolute methanol for 24 h at 4°C in darkness. This extract was centrifuged at 5,000 *g* for 10 min and then used to determine the amounts of UV-absorbing compounds and the photosynthetic pigments using a scanning spectrophotometer (UV 530; Beckman Coulter, USA). The contents of UVAC per fresh mass were estimated by determining the ratio of UVAC to chlorophyll *a* (Chl *a*) according to Dunlap et al. (1995) and multiplying the ratio of Chl *a* content to fresh weight of the sample used. Chl *a* concentration was estimated according to Wellburn (1994). For the determination of phycoerythrin, about 200 mg of thallus was ground thoroughly in 2 ml 0.1 M phosphate buffer (pH 6.8) with quartz sand, and then further extracted in 10 ml 0.1 M phosphate buffer for 0.5 h (pH 6.8). The extracts were centrifuged for 15 min at 5,000 *g* and the phycoerythrin concentration determined according to Beer and Eshel (1985).

Agar extraction

Thalli of *G. lemaneiformis* grown at different depths for about 45 days (from 20 March to 3 May 2004) were collected for the analysis of their agar content. Agar extraction was carried out according to Marinho-Soriano et al. (1999). Dried samples of about 5 g were immersed in 500 ml of distilled water (pH 6.0) in a flask and heated for 1h at 110°C. The extract was filtered at 70°C. The residue was re-extracted under the same conditions. The filtrates were gelled at room temperature and then frozen at −20°C overnight. The frozen gel was thawed, washed with distilled water and dried for 24 h at 60°C. The agar yield was calculated as the percentage of dry mass.

Evaluation of UVAC concentrations under indoor conditions

To examine the effect of low light in the absence of UVR on the contents of UVAC, an indoor experiment was carried out under very low PAR (~ 20 μmol photons m^{-2} s^{-1}) for 3 months (from 19 February to 16 May 2004). Thalli of about 10 g were maintained in a glass tank (~ 5 L) with filtered natural seawater. The contents of UVAC were

measured every 3 days for the first 2 weeks and at the end of the culture.

Incident solar irradiance was continuously monitored using a broad band filter radiometer (Eldonet; Real Time Computer, Möhrendorf, Germany), which has 3 channels respectively for photosynthetically active radiation (PAR, 400–700 nm), ultraviolet-A radiation (UV-A, 315–400 nm) and ultraviolet-B radiation (UV-B, 280–315 nm) (Häder et al. 1999). For underwater irradiances, a diving ELDONET was used. The daily dose of solar radiation was estimated at different depths in the testing periods. The solar PAR doses that the thalli received at different depths were estimated according to the measured diurnal solar irradiances and the PAR attenuation coefficients.

Statistical analysis

Differences among the treatments were tested using one-way analysis of variance (ANOVA) or *t*-test with SPSS (version 11.5). A confidence level of 95% was used in all analyses.

Results

From 20 March to 2 April 2004, the daily doses of solar PAR reaching the sea surface ranged 1 to 8.6 MJ m^{-2}, with the daily average of 4.04 MJ m^{-2}; while that of UVR ranged from 0.15 to 1.18 MJ m^{-2}, with the daily average of 0.57 MJ m^{-2} (Table 1). During this period, the thalli of *Gracilaria lemaneiformis* in the surface water (about 50 cm below the surface) grew fastest compared with greater depths to 3.5 m ($P<0.01$). The relative growth rate (RGR) in the surface water was 18 times higher than that at 2.5 m. At the depth of 3.5 m, negative RGR was observed, indicating that respiratory losses exceeded the photosynthetic gain (Fig. 1a, c).

From 11 to 21 April 2004, the daily solar PAR dose ranged from 2.9 to 11.8 MJ m^{-2} with the average value of 8.2 MJ m^{-2} per day, while that of UVR ranged from 0.48 to 1.76 MJ m^{-2}, with the average value of 1.27 MJ m^{-2} (Table 1). The thalli of *G. lemaneiformis* showed the highest growth rate at 1 m depth. The RGR at 0.5 m was 8% lower ($P<0.05$) than that at 1 m, reflecting a photoinhibition in the surface seawater caused by the high levels of solar radiation. Compared with the period at the end of March, *G. lemaneiformis* grew much faster in the middle of April at all depths ($P<0.01$) (Fig. 1b, c).

Despite the surface seawater temperature change, the RGR increased with increased daily solar PAR doses that the thalli received at different depths. Daily PAR dose of 4.3 MJ m^{-2} resulted the highest growth rate (Fig. 1d). At the highest levels of daily PAR of 5.8 MJ m^{-2} and UVR of 0.46 MJ m^{-2} the RGR decreased by 9.7%.

The content of both Chl *a* and phycoerythrin (PE) increased with increasing depth (Fig. 2). The highest contents of Chl *a* and PE were found at day 3, and the lowest at day 45 for depths of 1.5 m or less. At the deepest depth of 3.5 m, PE content was the highest at day 45, while Chl *a* reached similar level with days 3 and 21.

The content of UVAC at 0.5 m depth showed a significant ($P<0.05$) increase after 3 days in relation to the initial value, and reached the highest value at the end of the experiment. At other depths, the UVAC content decreased with increasing depth and with time and showed the lowest values at the end of the experiment. After 45 days, the UVAC concentrations of the thalli cultured at 0.5 m were higher ($P<0.05$) than that at days 3 and 21 (Fig. 3a, b). To test the change in UVAC content under indoor PAR without UVR, the thalli were cultured under low PAR (~20 μmol photons m^{-2} s^{-1}). The content of UVAC significantly deceased from 134.1±7.4 to 98.2±6.2 μg g^{-1}(f.w.) after 3 days ($P<0.01$), and then gradually decreased to a steady value of 51.2±4.3 μg g^{-1}(f.w.) after 2 weeks until the end of the culture period (90 days) (Fig. 3c).

Agar yield of *G. lemaneiformis* increased with the increasing depths (Fig. 4). The greatest agar yield was found in thalli cultured at 3.5 m (19.9±0.5%) and the least in the thalli cultured at 0.5 m (14.2±0.8%). There are

Table 1 Averaged daily doses of PAR, UV-A and UV-B during the growth periods of *Gracilaria lemaneiformis*

Daily dose	Depth (m)					
(MJ m^{-2})	0	0.5	1.0	1.5	2.5	3.5
March (20 March to 2 April 2004)						
PAR	4.04	1.34	0.45	0.15	0.016	0.002
UV-A	0.55	0.67	0.008	0.001	1.51×10^{-5}	2.27×10^{-7}
UV-B	0.015	6.51×10^{-4}	2.80×10^{-5}	1.20×10^{-6}	2.18×10^{-9}	4.00×10^{-12}
April (11 to 21 April 2004)						
PAR	8.24	5.78	4.05	2.84	1.40	0.69
UV-A	1.24	0.45	0.16	0.06	0.007	0.001
UV-B	0.036	0.014	0.005	0.002	3.13×10^{-4}	4.69×10^{-5}

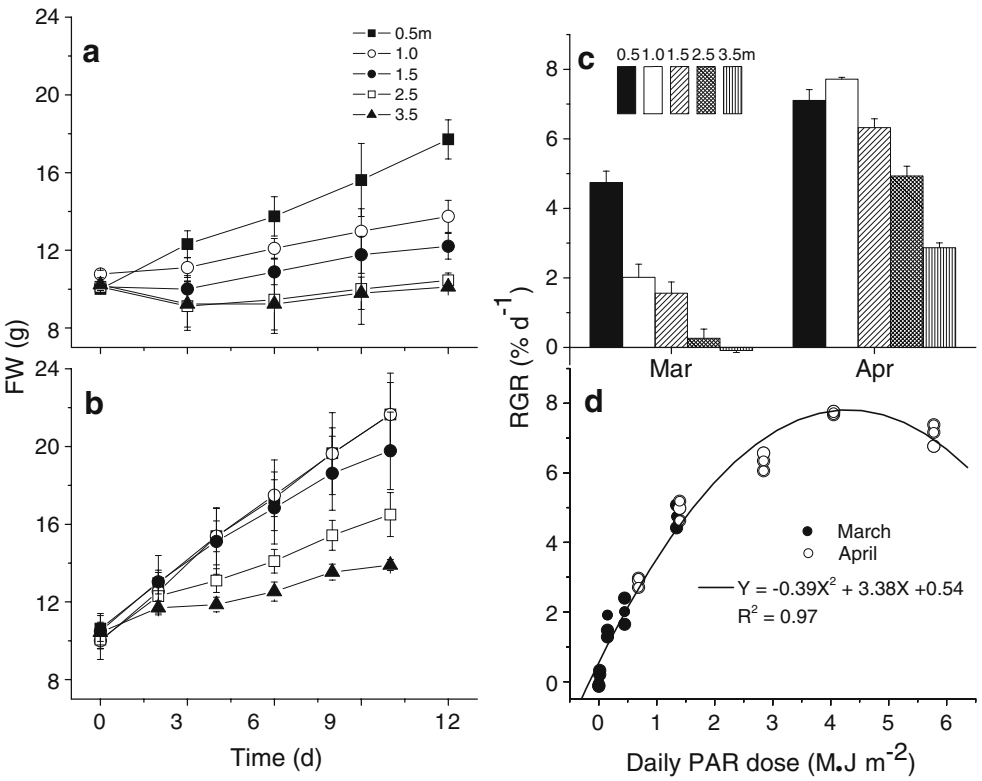

Fig. 1 Change in fresh biomass with time in *Gracilaria lemaneiformis* grown at different depths from 20 March to 2 April (**a**) and from 11 to 21 April 2004 (**b**), the relative growth rate (RGR) at different depths (**c**) and the relationship of the RGR with daily PAR dose (**d**). Daily PAR doses at deferent depths were calculated from the measured diurnal irradiances and the mean attenuation coefficients (*K*) for PAR wavebands. *Vertical bars* represent ± SD for the means (n=3)

significant differences (P<0.05) among depths except for the comparison between 1.0 and 1.5 m depths.

Discussion

Solar PAR, UV-A and UV-B attenuated disproportionatey with increasing depth. The averaged attenuation coefficients were 1.5 m^{-1} for PAR, 3.1 m^{-1} for UV-A and 4.1 m^{-1} for UV-B during the experimental periods, respectively. Solar irradiances were reduced to 1% of the sea surface at 2.3 m for PAR, 1.1 m for UV-A and 0.3 m for UV-B during the March experiment and at 6.5 m for PAR, 2.3 m for UV-A and 2.4 m for UV-B during the April culture. Our results showed that the growth rate of *G. lemaneiformis* was closely related to the daily PAR dose, while UVR showed insignificant effects on the growth except for the thalli grown at surface water during the April experiment. High levels of UVR at surface water during this period could be responsible for the observed decrease in the RGR, since UV-B is known to damage DNA (Zacher et al. 2007) and reduce photosynthetic capacity (Hanelt et al. 1997; Han et al. 2003) in macroalgae. On the other hand, *G. lemaneiformis* had higher Chl *a* and PE contents at deeper depth. Cells grown at deeper water layers can make more efficient use of low irradiances by increasing the contents of photosynthetic pigments (Malta et al. 2003), but

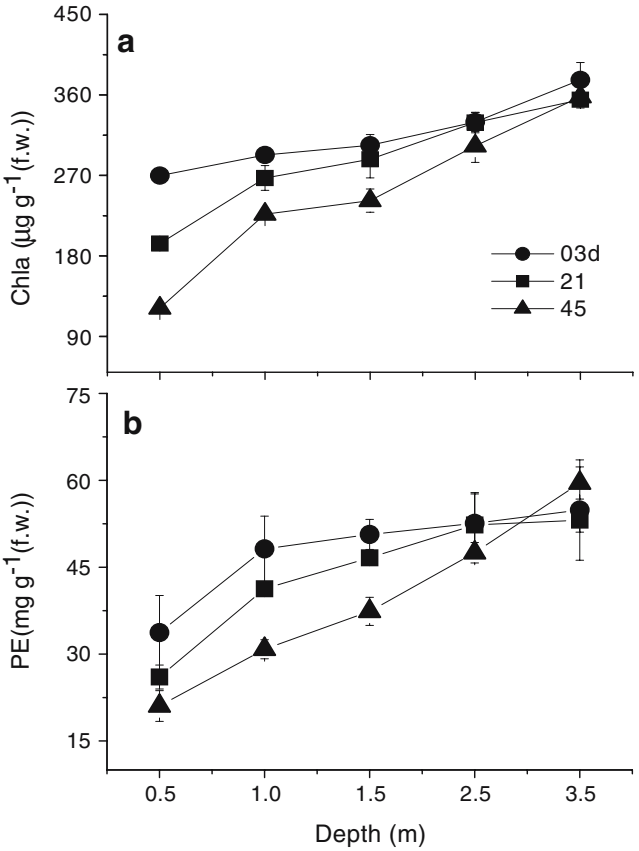

Fig. 2 The contents of Chl *a* (**a**) and PE (**b**) in *G. lemaneiformis* thalli grown at different depths during the period of 19 March to 3 May 2004. *Vertical bars* represent ± SD for the means (n=3)

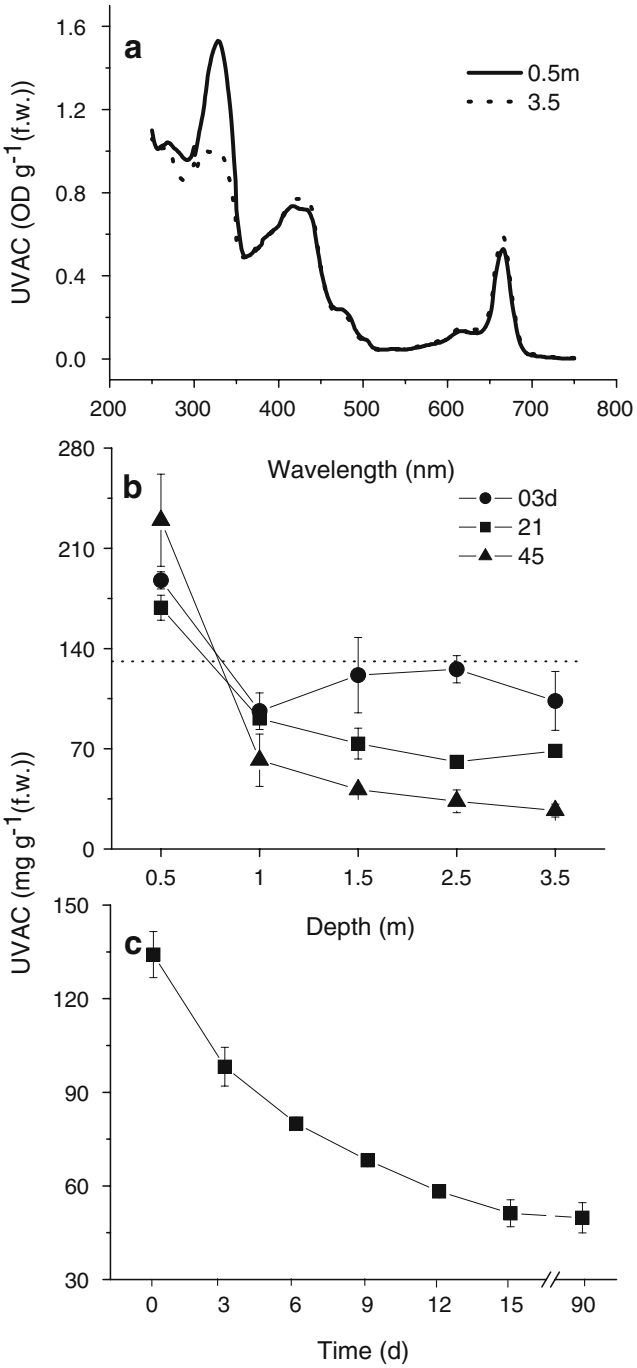

Fig. 3 Absorption spectra of *G. lemaneiformis* thalli grown at 0.5 and 3.5 m depths after 21 days (**a**), the contents of UVAC in the thalli at different depths (**b**) and the changes in the UVAC content of the thalli grown under low PAR from 19 February to 16 May 2004. *Dotted line* indicates the initial level of UVAC concentration. *Vertical bars* represent ± SD for the means (n=3)

severe shortage of light at deeper depths can still result in reduction of growth rate as shown in our study.

At the surface water or shallower layers where UVR may result in harmful effects, *G. lemaneiformis* thalli accumulated higher concentrations of UVAC. Algae inhabiting shallow water are more likely to contain higher levels of UVAC which plays a protective role against UVR (Maegawa et al. 1993; Karsten et al. 1998). In the present study, the thalli grown under indoor low PAR without UVR had decreased contents of UVAC in about 2 weeks, indicating that decreased UVAC contents at deeper depths were related to the lower solar irradiance and the negligible amount of UVR.

Gracilaria lemaneiformis responded to reduced solar radiation at increased depths by increasing cellular contents of the photosynthetic pigments, Chl *a* and PE and decreasing the content of UVAC. This strategy can enable the alga to capture more light for photosynthesis and to reduce the metabolic cost of the production of the secondary products, UVACs. Because formation of secondary products and cell growth are antagonistic processes and substantial metabolic investment might be required for the production of UV-absorbing compounds (Raven 1991), the decrease of UVAC concentrations can reduce the metabolic investment and ensure the limited photosynthetic products are preferentially provided for growth.

High levels of UV-B can reduce production of agar (Eswaran et al. 2002). In the present study, we observed the lowest agar yield in thalli grown in surface water, and the agar content increased with depth. However, at depths of 2.5 or 3.5 m, where the UVR was negligible (less than 1% of the surface UVR), agar yield was significantly higher than at other depths. While UVR may negatively affect agar yield in shallower layers, decreased growth rate at greater depths might contribute to the accumulation of agar due to reduced cell division.

Gracilaria lemaneiformis grew fastest in surface seawater in March and at 1 m depth in April. This implied that, in the coastal waters of the South China Sea, *G. lemaneiformis* would be better grown in surface seawater in February and

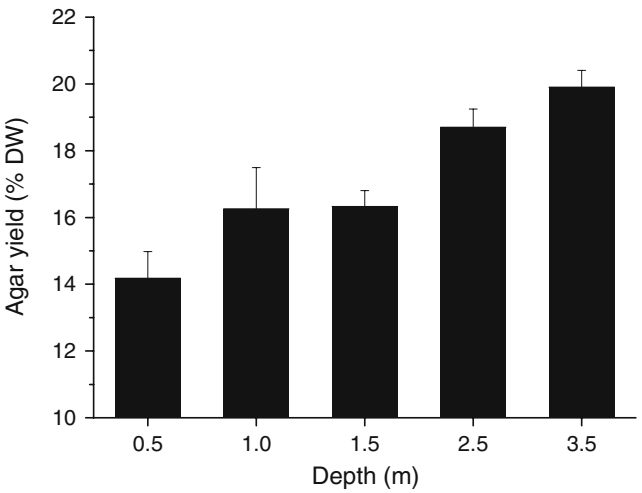

Fig. 4 The agar yield, as percentage of the dry mass of *G. lemaneiformis* grown at different depths. *Vertical bars* represent ± SD of the means (n=3)

March and at a depth of about 1 m in April and May, when solar radiation is high. The results of this study can help us understand to what extent changes in solar PAR or UVR can affect the growth, pigments and agar yield of *G. lemaneiformis*. Stocking density may also have and important effect on growth rate since it affects the intensity of either PAR or UVR. In view of the RGR–PAR dose relationship (Fig. 1d), increased stocking density will certainly reduce the optimum growth depth

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