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IMPACTS OF SOLAR UV RADIATION ON THE PHOTOSYNTHESIS, GROWTH, AND UV-ABSORBING COMPOUNDS IN *GRACILARIA LEMANEIFORMIS* (RHODOPHYTA) GROWN AT DIFFERENT NITRATE CONCENTRATIONS[1]

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Solar ultraviolet radiation (UVR, 280–400 nm) is known to affect macroalgal physiology negatively, while nutrient availability may affect UV-absorbing compounds (UVACs) and sensitivity to UVR. However, little is known about the interactive effects of UVR and nitrate availability on macroalgal growth and photosynthesis. We investigated the growth and photosynthesis of the red alga *Gracilaria lemaneiformis* (Bory) Grev. at different levels of nitrate (natural or enriched nitrate levels of 41 or 300 and 600 μM) under different solar radiation treatments with or without UVR. Nitrate-enrichment enhanced the growth, resulted in higher concentrations of UVACs, and led to negligible photoinhibition of photosynthesis even at noon in the presence of UVR. Net photosynthesis during the noon period was severely inhibited by both ultraviolet-A radiation (UVA) and ultraviolet-B radiation (UVB) in the thalli grown in seawater without enriched nitrate. The absorptivity of UVACs changed in response to changes in the PAR dose when the thalli were shifted back and forth from solar radiation to indoor low light, and exposure to UVR significantly induced the synthesis of UVACs. The thalli exposed to PAR alone exhibited higher growth rates than those that received PAR + UVA or PAR + UVA + UVB at the ambient or enriched nitrate concentrations. UVR inhibited growth approximately five times as much as it inhibited photosynthesis within a range of 60–120 μg UVACs · g^{-1} (fwt) when the thalli were grown under nitrate-enriched conditions. Such differential inhibition implies that other metabolic processes are more sensitive to solar UVR than photosynthesis.

***Key index words:* *Gracilaria lemaneiformis*; growth; nitrate; photosynthesis; solar ultraviolet radiation; UV-absorbing compounds**

***Abbreviations:* fwt, fresh weight; PA, PAR + UVA (320–700 nm); PAB, PAR + UVA + UVB (280–700 nm); PAR, photosynthetically active radiation (400–700 nm); Pn, net photosynthesis; RGR, relative growth rate; SW + 300, seawater enriched with 300 μM nitrate; SW + 600, seawater enriched with 600 μM nitrate; SW, seawater; UVACs, UV-absorbing compounds; UVR, UVA + UVB (280–400 nm)**

Marine macroalgae play an important role in coastal ecosystems adjacent to populated areas and provide useful resources for food and potential biofuels (Gao and Mckinley 1994). Their physiological behaviors respond to many environmental changes (Altamirano et al. 2000a), such as solar radiation (Hanelt et al. 1997, Häder et al. 2001), temperature (Todd and Lewis 1984, Rautenberger and Bischof 2006), salinity (O'Neal and Prince 1988, Kamer and Fong 2001), heavy metals (Gledhill et al. 1997), and even atmospheric CO_2 concentrations (Gao et al. 1991, 1993). Solar UV radiation (280–400 nm) is a permanently existing environmental factor that macroalgae usually experience. Natural levels of UVR have been determined to affect activities of enzymes (Flores-Moya et al. 1998, Bischof et al. 2002); damage DNA (Roleda et al. 2006a); reduce growth rate (Altamirano et al. 2000b, Pang et al. 2001, Michler et al. 2002, Roleda et al. 2006a); and inhibit photosynthesis (Hanelt et al. 1997, Brouwer et al. 2000, Dring et al. 2001, Zacher et al. 2007), spore germination (Wiencke et al. 2000, Han et al. 2004, Roleda et al. 2006b), early development (Dring et al. 1996, Huovinen et al. 2000, Henry and Van-Alstyne 2004, Jiang et al. 2007, Wiencke et al. 2007), and even community structure (Bischof et al. 2006) of macroalgae.

Macroalgae have developed mechanisms to counteract the damaging effects of UVR through their evolutionary history. A known mechanism of protection involves the synthesis of UVACs. These compounds absorb and lessen the damaging effects of UVR exposure (Oren and Gunde-Cimerman 2007). UVACs with maximal absorption spectra

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within 310–360 nm (Nakamura et al. 1982), mainly mycosporine-like amino acids (MAAs) (Karentz et al. 1991), are regarded as effective sunscreens against harmful UVR (Karsten and Wiencke 1999, Shick and Dunlap 2002). Under solar UV radiation, macroalgae often exhibit higher levels of UVACs, such as the MAAs in red algae (Karsten et al. 2000), phlorotannin in brown algae (Pavia et al. 1997), and uncharacterized UVACs in green algae (Han and Han 2005). Concentrations of UVACs are usually affected by environmental factors such as light (Karsten et al. 1998) and nutrient availability (Korbee-Peinado et al. 2004), whereas nutrient concentrations can also influence the extent of photoinhibition elicited by UVR treatment. Nitrogen is regarded as a limiting nutrient for the primary productivity of macroalgae in coastal ecosystems (Lobban and Harrison 1994). N-limitation was shown to decrease the growth rate of macroalgae (Larned and Atkinson 1997) and increase the rate of UVR-induced photoinhibition in a dinoflagellate (Litchman et al. 2002). Repair of damaged DNA by photoreactivation (Pakker et al. 2000a) and enzymatic detoxification of oxygenic radicals (Foyer et al. 1994) are also important mechanisms for ameliorating the effects of UVR on macroalgae.

G. lemaneiformis has been farmed in coastal waters of northern and southern parts of China for food and agar production. The concentration of nutrients, including nitrate, in the Chinese coastal waters varies with seasons and sites due to the inputs from terrestrial runoff or aquaculture activities. Therefore, it is of general interest to know how the photosynthesis and growth of this alga are affected by solar UVR under changing nutrient concentrations. We report here the combined effects of different concentrations of nitrate and solar radiation on this species.

MATERIALS AND METHODS

Algal material and culture. *G. lemaneiformis* farmed in surface seawater was collected from Nan'ao Island (23.3° N, 116.6° E), Shantou, Guangdong, China, in May 2005 and May 2008, when surface seawater temperature (SST) ranged from 22.2°C to 25.7°C. The concentrations of nitrate during the experiment periods varied within a range of 41.5–43.1 μM, from 18 May to 2 June 2005 and varied between 40.2 and 41.1 μM from 1 to 10 May 2008. Branches (10–15 cm long) of about 2 g (fwt) from different individuals were fixed on stainless steel wires and placed in a quartz tube (Φ 4 cm, 35 cm long, about 0.44 L). The biomass density in the tubes was maintained at about 100 g (fwt) $\cdot$ m^{-2} to avoid self-shading [biomass density ranged from 100 to 5,000 g (fwt) $\cdot$ m^{-2} in the farmed area]. The tubes with the thalli were placed in a water tank with surface seawater running through to keep the temperature similar to the SST. The cultures were aerated with ambient air at a rate of 0.5 L $\cdot$ min^{-1}.

Nitrate enrichment. The nitrate concentrations for the ambient (sand-filtered) natural seawater (SW) are described above. Enriched nitrate levels were set as SW plus 300 μM nitrate (SW + 300) and SW plus 600 μM nitrate (SW + 600), respectively. The culture media were renewed with nitrate every 24 h. For the enriched nitrate concentrations, 0.102 or 0.204 g sodium nitrate was added to 4 L natural filtered seawater and stirred to dissolve completely.

Solar radiation treatments and monitoring. The thalli were exposed to different solar radiation treatments: (1) full spectrum solar radiation (PAB treatment), uncovered quartz tubes; (2) PAR + UVA (PA treatment), quartz tubes covered with Folex 320 (Montagefolie, Nr. 10155099, Folex, Dreieich, Germany), which transmits solar radiation >320 nm; and (3) PAR alone (PAR treatment), quartz tubes covered with Ultraphan film 395 (UV Opak, Digefra, Munich, Germany), which transmits solar radiation >395 nm. The transmission spectra of these cutoff foils are shown in Figure 1. For the experiments in May 2005, only two radiation treatments (PAR and PAB) were employed, and in May 2008, measurements were carried out under the three different radiation treatments described above to distinguish the effects of UVA from UVB. The uncovered quartz tubes received 4% higher PAR compared with the covered tubes due to the reflection by the foil under water (Gao et al. 2007b). Incident solar radiation was continuously monitored using a broadband ELDONET filter radiometer (RealThinClient, Frankfurt/Main, Germany) with three channels, for PAR (400–700 nm), UVA (315–400 nm), and UVB (280–315 nm), respectively (Häder et al. 1999). This instrument has been internationally recognized and certificated (Häder et al. 1999), with an error of <0.5% in comparison with the most accurate instrument (certificate No. 2006/BB14/1). The instrument has been calibrated annually with the assistance of the manufacturer. The instrument was placed at the marine biological station of Shantou University located on Nan'ao Island (23.3° N, 116.6° E). Since there was 5 nm difference between the measured and exposed irradiance for UVA, the cells received about 2% less than the measured amount of UVA.

Measurement of growth. While the thalli (between three and five in each tube, and three tubes for each treatment) were cultured at different NO_3^- concentrations under radiation treatments of PA, PAB, or PAR (no UVR) for a period of 16 d in 2005 (May 18 to June 2) or for 10 d in 2008 (May 1 to 10), changes in fresh weight were assessed every 4 or 2 d. The fresh weight was determined after blotting water drops slightly with tissue paper. Relative growth rate (RGR, % $\cdot$ d^{-1}) was estimated as follows: $RGR = 100 * (\ln N_t - \ln N_0)/t$, where N_0 and N_t represent the fresh weight at the beginning or end of the measuring interval, respectively.

Determination of UVACs. About 0.2 g (fwt) thalli was ground and extracted in 10 mL absolute methanol at 4°C in darkness for 24 h (Gao and Xu 2008). After centrifugation at 5,000*g* for

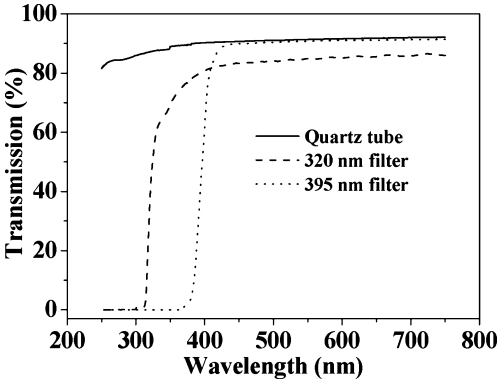

FIG. 1. Transmission spectra of the cutoff foils and quartz tubes used in the experiments.

10 min, the absorbance of the supernatant was measured in the range of 250–750 nm using a scanning spectrophotometer (UV 530; Beckman Coulter, Fullerton, CA, USA), and the absorption peak at 325 nm was used to estimate total absorptivity of UVACs relative to chl *a* according to Dunlap et al. (1995). The concentration of chl *a* was determined according to Wellburn (1994), and the absorptivity of UVACs per fresh weight normalized to the level of chl *a*.

Determination of photosynthetic oxygen evolution. A closed system modified from Gao and Umezaki (1989) for measuring photosynthesis in running seawater was used. Thalli fixed on stainless steel wires with a biomass density of ~40 g (fwt) · m^{-2} (without self-shading) were placed in a quartz tube (Φ5 cm, 59 cm long, ~1.2 L). The flow rate of seawater was maintained by a peristaltic pump (01268-16; Cole-Parmer Instrument Co., Vernon Hills, IL, USA) at 0.6 L · min^{-1}. Dissolved oxygen in the circulated seawater as it passed through the quartz tube was monitored with a Clark-type oxygen microelectrode (YSI model 5300; Yellow Spring, OH, USA) interfaced with an oxymeter (Oxym 5; RealThinClient) connected to a laptop computer. The net photosynthetic rate was determined from the linear change in the dissolved oxygen concentration over a period of about 2 min as follows: $Pn = \Delta O_2 \times V \times 1/W \times 1/t$, where ΔO_2 represents the difference in the dissolved oxygen concentration (μmol · L^{-1}) during the measuring period of t (min); V, the seawater volume (L) in the closed system; and W, fresh weight (g) of the sample. The photosynthetic rates under PAB or PAR treatments were determined on 21, 25, and 29 May, and 2 June 2005 around noon (10:00–14:00 h). For the 10 d experiment in May 2008, the photosynthetic rate was determined under PA, PAR, and PAB conditions using the same procedures as in 2005.

Determination of UVR inhibition. The relative inhibition caused by UVR was estimated as: $(P_{PAR} - P_{PAB}) * (P_{PAR})^{-1}$, where P_{PAR} and P_{PAB} represent the growth or photosynthetic rate of the thalli exposed to PAR only or PAR + UVA (PA) or PAR + UVA + UVB (PAB), respectively.

Test of PAR and UVR-related impacts on the UVACs. To determine how changes in the levels of PAR and UVR affect the absorptivity of UVACs, the thalli of *G. lemaneiformis* grown at the three levels of nitrate were shifted from indoor to outdoor conditions during the period 1–24 August 2005, when the mean temperature of seawater was about 26.1°C. The quartz tubes containing thalli, either uncovered (PAB treatment) or covered (PAR treatment) with the UV-cutoff foil were moved back and forth from the indoor low light (natural scattered sunlight by the window, with a maximum PAR of 20 μmol · m^{-2} · s^{-1} during noontime; the window glass allowed <8% of solar UVR to penetrate) to solar radiation ever 4 d. The temperature control, renewal of medium, and aeration were the same as mentioned above.

Statistical analysis. One-way analysis of variance (ANOVA) and Tukey's test were used to establish differences among treatments (Origin 7.0, v7.0220 [B220]; OriginLab Corp., Northampton, MA, USA). A confidence level was set at 95%.

RESULTS

During the period from May 18 to June 2, 2005, when *G. lemaneiformis* thalli were grown in the presence of different levels of NO_3^- and solar radiation, the daily solar doses varied in the ranges of 0.4–13.2 MJ · m^{-2} for PAR, 0.08–2.2 MJ · m^{-2} for UVA, and 0.002–0.06 MJ · m^{-2} for UVB (Fig. 2A). SST fluctuated with that of solar radiation. During the period from May 1 to 10, 2008, the daily solar doses, however, varied in the ranges of 1.4–8.4 MJ · m^{-2} for PAR, 0.2–0.9 MJ · m^{-2} for UVA, and 0.006–0.03 MJ · m^{-2} for UVB.

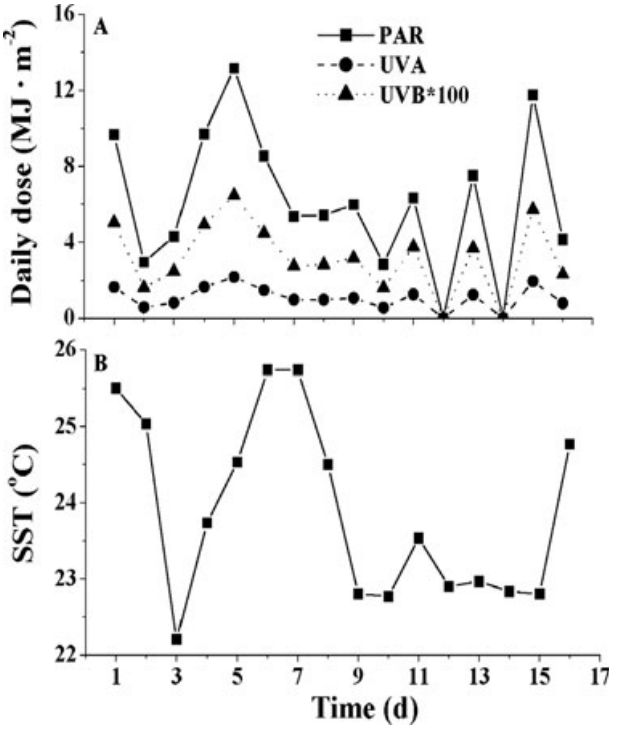

FIG. 2. Changes of daily solar doses of PAR, UVA, and UVB (A) and surface seawater temperature (SST) of natural seawater (B) during the experiment, which lasted from May18 to June 2.

For the results obtained in 2005, under the PAR treatment, additions of 300 μM NO_3^- to SW (SW + 300, 341–343 μM) increased the growth rate relative to SW (RGR) by 146.1%–199.6%, while enrichment to 641–643 μM (SW + 600) raised RGR by 214.1%–283.1%, (Fig. 3). The mean RGR values per day under PAR treatment throughout the period were 4.3 ± 0.5 in SW, 11.4 ± 1.0 in SW + 300, and 14.1 ± 0.5 in SW + 600, respectively. The RGR was significantly higher in the NO_3^--enriched SW relative to SW ($P < 0.0001$), with the SW + 600 having the highest RGR. Under PAB treatment, the RGR was significantly lower ($P < 0.01$) compared with PAR treatment, reflecting an inhibitory effect of UVR. In the seawater without the NO_3^- enrichment (SW), UVR-induced inhibition was about 43.6% on the fourth day, 36.4% on the eighth day, 36.0% on the 12th day, and 34.5% on the 16th day, respectively (Fig. 3). In the NO_3^--enriched seawater, UVR-induced inhibition ranged from 20.2% to 22.4% in SW + 300 and 17.7% to 20.8% in SW + 600, respectively (Fig. 3); the inhibition is significantly ($P < 0.001$) less than that observed in the unsupplemented SW. The mean RGR per day under the PAB treatment was 2.7 ± 0.4 in SW, 8.9 ± 0.7 in SW + 300, and 11.4 ± 0.6 in SW + 600, respectively (Fig. 3). In the presence of UVR, the RGR was also

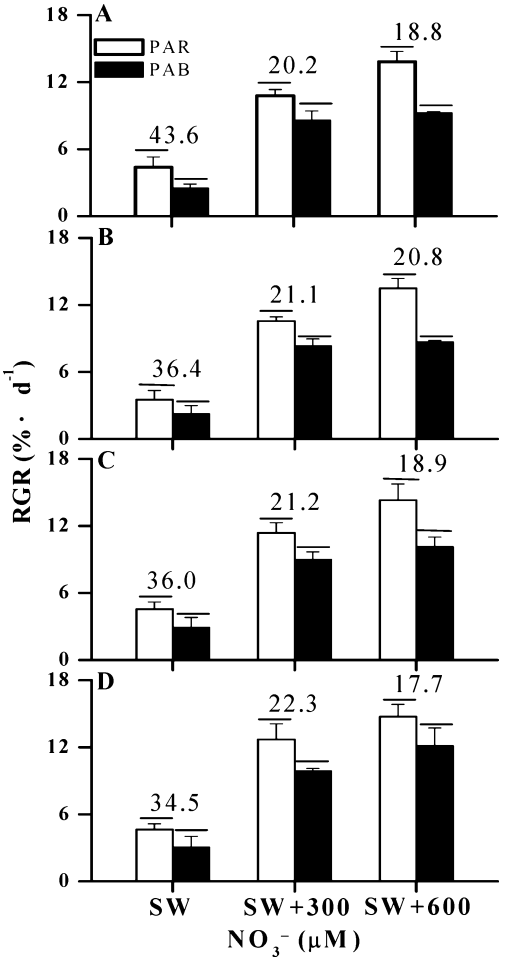

FIG. 3. The relative growth rate (RGR) of *Gracilaria lemaneiformis* thalli in seawater treated with different concentrations of nitrate under full spectrum of solar radiation (PAB) and PAR alone (P). (A, B, C, D) RGR at the fourth day, the eighth day, the 12th day, and the 16th day for the culture period from May 18 to June 2, 2005, respectively. Data are the means ± SD ($n = 3$). The horizontal lines over the bars indicate significant differences between treatments. Data on the top of the bars represent the percentage of inhibition of growth caused by UVR. SW, seawater; SW + 300, seawater enriched with 300 μm nitrate; SW + 600, seawater enriched with 600 μm nitrate.

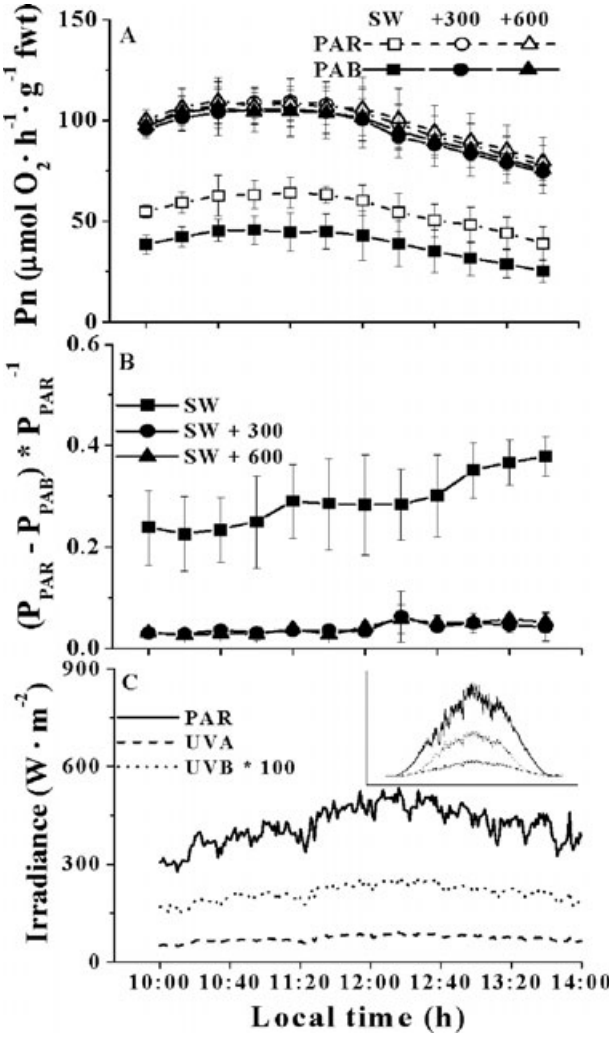

FIG. 4. Photosynthetic oxygen evolution rates around noon (10:00–14:00 h) (A) and UV-induced inhibition (B) of *Gracilaria lemaneiformis* thalli grown under different levels of nitrate under full spectrum of solar radiation (PAB) or PAR alone over the days 21, 25, and 29 May and 2 June 2005. Averaged solar irradiances of PAR, UVA, and UVB during the measuring period over the days are shown in (C). The photosynthetic rates are the means ± SD ($n = 12$) over the 4 d. SW, seawater; SW + 300, seawater enriched with 300 μm nitrate; SW + 600, seawater enriched with 600 μm nitrate.

significantly higher ($P < 0.0001$) in the thalli grown in NO_3^--enriched seawater than that in SW, while the difference between SW + 300 and SW + 600 was also significant ($P < 0.005$).

The rates of photosynthetic oxygen evolution measured during the noon period (10:00–14:00) under the solar radiation treatments with or without UVR are shown for the thalli grown at the different NO_3^- levels (Fig. 4). Irradiances averaged over the measuring periods on 21, 25, and 29, May and 2 June 2005 were 420.4 W · m^{-2} for PAR, 73.3 W · m^{-2} for UVA, and 2.6 W · m^{-2} for UVB, respectively. Net photosynthesis (Pn) ranges were 38.9–64.1 in SW, 77.6–109.6 in SW + 300, and 79.7–108.7 μmol $O_2 \cdot h^{-1} \cdot g^{-1}$ (fwt) in SW + 600, respectively, when thalli received PAR alone (Fig. 4A). The enrichment of NO_3^- enhanced the Pn by 79.0%–81.7% on average. The Pn was significantly higher ($P < 0.001$) in the NO_3^--enriched seawater relative to SW, while the difference between SW + 300 and SW + 600 was not significant ($P > 0.7$). When the thalli were exposed to the full spectrum of solar radiation (PAB treatment), Pn was significantly lower ($P < 0.001$) compared with that under PAR alone in SW; however, such a difference was not found ($P > 0.7$) under the NO_3^--enriched treatments. The values for Pn under PAB treatment were 38.6 ± 6.9 in SW, 94.4 ± 10.9 in SW + 300, and 95.6 ± 10.5 μmol $O_2 \cdot h^{-1} \cdot g^{-1}$ (fwt) in SW + 600, respectively. Exposure to UVR inhibited

the Pn by 22.5%–37.8% in SW, 2.9%–4.2% in SW + 300, and 2.7%–4.2% in SW + 600, respectively (Fig. 4B).

The effects of UVA or UVB on the growth and photosynthesis of *G. lemaneiformis* were respectively examined in the experiments carried out in May 2008 (Fig. 5). The mean RGR per day in SW was 6.9 ± 0.6 under PAR, 6.2 ± 0.3 under PA, and 4.8 ± 0.4 under PAB; that in SW + 300 was 13.1 ± 2.0 under PAR, 11.9 ± 1.2 under PA, and 9.9 ± 0.8 under PAB, respectively. The rates of net photosynthetic O_2 evolution (Pn) in SW (SW+300) were 61.3 ± 1.6 (109.8 ± 1.3) under PAR, 60.6 ± 1.7 (108.9 ± 1.1) under PA, and 41.8 ± 0.5 (103.4 ± 0.7) μmol $O_2 \cdot h^{-1} \cdot g^{-1}$ (fwt) under PAB, respectively. UVA and UVB inhibited the RGR by 10.1% and 20.3% in SW and by 9.1% and 15.3% in SW + 300 (Fig. 5) and reduced the Pn by 1.1% and 30.7% in SW and by 0.8% and 5.0% in SW + 300, respectively. The difference in RGR or Pn among the radiation treatments was significant ($P < 0.05$) between the PAR and PAB or the PA and PAB treatments; however, it was insignificant between P and PA treatments ($P > 0.3$), reflecting a harmless effect of UVA (Fig. 5). When comparing the data on RGR or Pn obtained in the two different years, no significant ($P > 0.2$) difference was found under the same treatments.

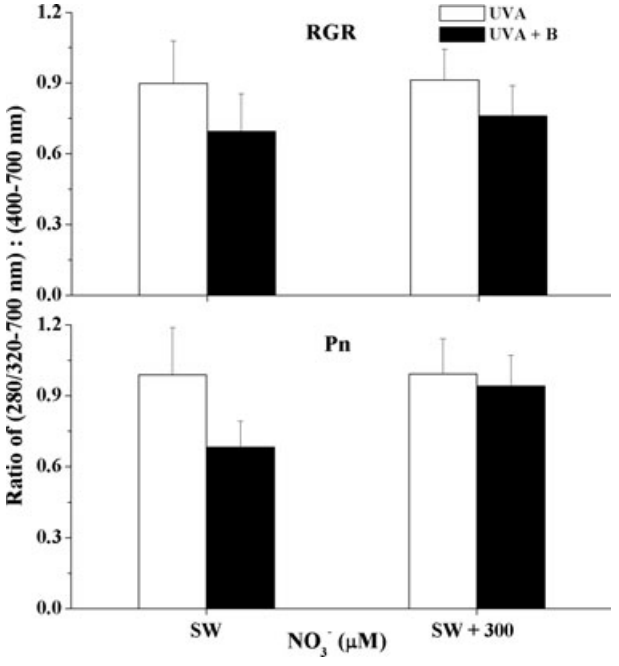

FIG. 5. The relative growth rate (RGR) and net photosynthesis (Pn) of *Gracilaria lemaneiformis* exposed to PAR + UVA + UVB (280–700 nm) or PAR + UVA (320–700 nm) as compared to exposure to PAR alone (400–700 nm). The ranges of daily doses of PAR, UVA, and UVB during the experimental period from May 1 to 10, 2008, were 1.4–8.4, 0.2–0.9, and 0.006–0.03 MJ $\cdot m^{-2}$, respectively. Photosynthetic O_2 evolution was determined during noontime at day 10. The data, representative of RGR, were the means ± SD of five individuals, respectively, measured on 2, 4, 6, 8, and 10 May 2008 ($n = 25$), which for Pn were for nine different individuals in three measurements ($n = 9$). Absolute values of the RGR or Pn are shown in the text. SW, seawater; SW + 300, seawater enriched with 300 μm nitrate.

UVACs with absorption peaking at 325 nm increased with time either under PAB or PAR treatments (Figs. 6 and 7), as indicated for days 4 and 16. Regardless of the radiation treatments, enrichments of NO_3^- significantly ($P < 0.0001$) increased the absorptivity of UVACs (Fig. 7). The NO_3^- enrichments raised the absorptivity of UVACs by 37.4% under PAR and by 44.8% under PAR + UVR (PAB) (Fig. 6). Presence of UVR resulted in significant ($P < 0.001$) increase of the UVACs at any level of the NO_3^- concentrations (Fig. 6). However, the difference between the two NO_3^--enriched levels was not significant ($P > 0.6$). Compared with that under PAR treatment, the content of chl *a* in the thalli exposed to PAR+UVR decreased by 15.1% in SW, 10.2% in SW+300, and 10.1% in SW+600, respectively. The ratio of the integrated absorption areas of UVAC to that of chl *a* (Fig. 6) ranged from 0.47 to 0.44 under PAR; however, under PAB, the ratio was within the range of 0.81–0.85, reflecting a difference caused by UVR, which decreases chl content and enhances synthesis of UVAC.

The UVACs absorptivity increased (or decreased) with increased (or decreased) solar radiation, while the thalli were switched from the low (indoor) to the high solar radiation (outdoor) or vice versa (Fig. 8). The ratio of UVAC absorptivity under PAR + UVR to PAR alone was higher ($P < 0.01$) under direct solar radiation than under reduced scattered light. Presence of UVR significantly ($P < 0.0001$) enhanced the synthesis of UVACs, while enriched NO_3^- decreased the ratio by about 3% (Fig. 8), reflecting a reduction of the UVR-induced effect.

When the UVR-induced inhibition of growth and photosynthesis was analyzed as a function of the absorptivity of UVACs, it was clearly indicated that increased absorptivity of UVACs led to lower inhibition caused by UVR (Fig. 9). However, when the UVAC-dependent inhibition was compared between growth and photosynthesis, UVR caused much higher inhibition on growth than photosynthesis. At the lowest UVAC level of about 41 μg $\cdot g^{-1}$ (fwt), UVR caused 44% and 35% reduction of growth and photosynthesis, respectively. However, at the highest UVAC absorptivity of about 102 μg $\cdot g^{-1}$ (fwt), UVR induced 20% and 4% decline in growth and photosynthesis, respectively. The difference between UVR-induced inhibition between photosynthesis and growth was significant ($P < 0.0001$) when UVACs absorptivity exceeded 45 μg $\cdot g^{-1}$ (fwt). UVR-induced inhibition on growth was about five times that of photosynthesis within the UVAC absorptivity range of 60–102 μg $\cdot g^{-1}$ (fwt) when the thalli were grown under the nitrate-enriched conditions.

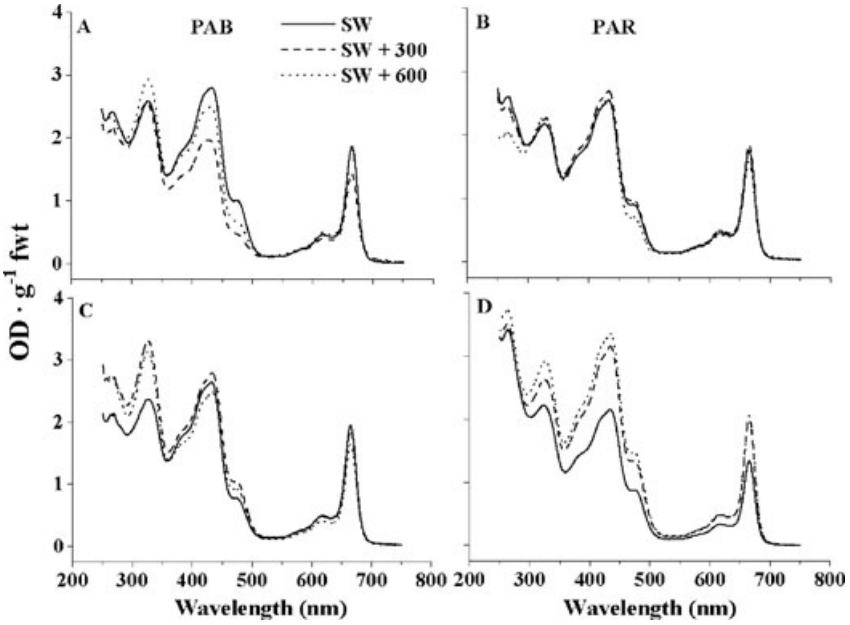

FIG. 6. Absorptivity of the methanolic extracts from the thalli of *Gracilaria lemaneiformis* grown under different nitrate concentrations and solar radiation treatments. (A, B) The UVAC absorptivity for the fourth day. (C, D) The spectra for the 16th day. Data are the means of triplicate samples. SW, seawater; SW + 300, seawater enriched with 300 μm nitrate; SW + 600, seawater enriched with 600 μm nitrate; UVAC, UV-absorbing compounds.

DISCUSSION

The growth, absorptivity of UVACs, and photosynthesis of *G. lemaneiformis* were enhanced with enriched NO_3^- in seawater under the radiation treatments with or without UVR. NO_3^--enrichment resulted in higher absorptivity of UVACs that led to decreased inhibition caused by UVR in both growth and photosynthesis. However, UVR-induced inhibition of growth was much higher than that of photosynthesis even at the highest levels of UVACs (Fig. 9). Such a differential inhibition implies that other metabolic processes can be more sensitive to solar UVR than photosynthesis and that the accumulation of UVACs was inadequate to protect the alga from the harm caused by UVR. Although both UVA and UVB wavebands have been reported to contribute to the accumulation of the UVACs (Kräbs et al. 2002), most of the inhibition caused by UVR in photosynthesis or growth rate was due to UVB (Fig. 5).

UVR is known to damage the proteins of PSΠ (Olsson et al. 2000), affect photochemical efficiency (Gao et al. 2007a), reduce the growth rate (Roleda et al. 2006a), and even induce production of oxidative species, such as $^1O_2$ (Shiu and Lee 2005). Presence of solar UVR in the present study reduced the RGR of *G. lemaneiformis* by as much as 37%, comparable to 31% reported for the brown alga *Dictyota dichotoma* (Kuhlenkamp et al. 2001) and 32% for the red alga *Porphyra haitanensis* (Jiang et al. 2007). In the present study, UVA caused much lower inhibition than UVB in both growth and photosynthesis (Fig. 5). When the solar dose was low, UVA was determined to benefit photosynthesis of *G. lemaneiformis* (Gao and Xu 2008) as well as phytoplankton (Gao et al. 2007b). Decreased inhibition of the growth at elevated NO_3^- concentrations could be attributed to reduced damage or increased repairing rate for damaged molecules (Litchman et al. 2002), which are key to photosynthesis and other metabolic processes (Viñegla et al. 2006). Increased NO_3^- levels led to an increase in absorptivity of UVACs, which appeared to play an important role in protecting the cell from the damaging effects of UVR, especially with respect to the activity of processes such as photosynthesis (Fig. 4B). However, the improved photosynthesis did not proportionally improve the growth, which was still reduced by UVR at enhanced levels of UVACs or NO_3^-. Photoprotective strategies against UVR must have competed with growth for nitrogen. NO_3^- enrichment can support higher rates of protein turnover by supplying sufficient nitrogen for the higher demand in repairing the damage caused by UVR.

Ammonium is always absorbed in preference to nitrate when both of them are available, and the enrichment of nitrate might result in a slightly higher rate of O_2 evolution due to the nitrate reduction (Stumm and Morgan 1996). In the present study, ammonia was present at about 2 μM. Enrichment of NO_3^- enhanced the photosynthetic O_2 evolution by 79.0%–81.7%, which can hardly be

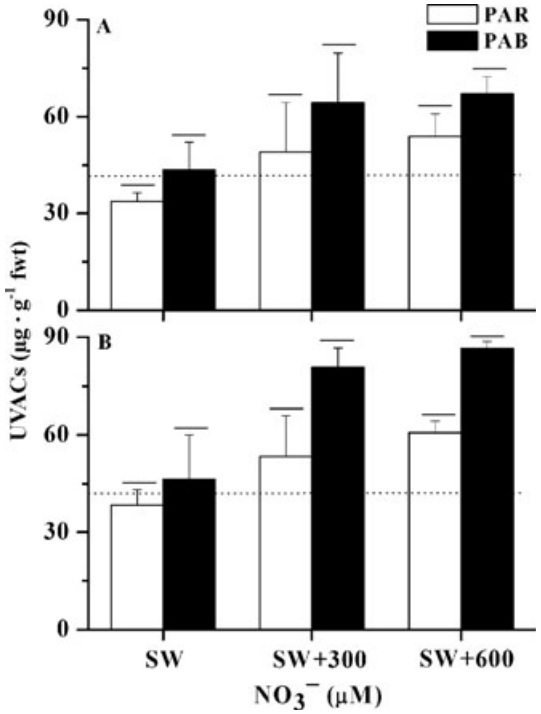

FIG. 7. Absorptivity of the UV-absorbing compounds (UVACs) of *Gracilaria lemaneiformis* thalli grown under the same treatments and over the same period as mentioned in Figure 2. (A, B) The levels of UVACs at the fourth day and the 16th day of the culture time (from May 18 to June 2, 2005), respectively. Data are the means ± SD ($n = 3$). The horizontal lines over the bars indicate significant differences between the treatments, and the dashed line represents the initial UVAC concentrations. SW, seawater; SW + 300, seawater enriched with 300 μm nitrate; SW + 600, seawater enriched with 600 μm nitrate.

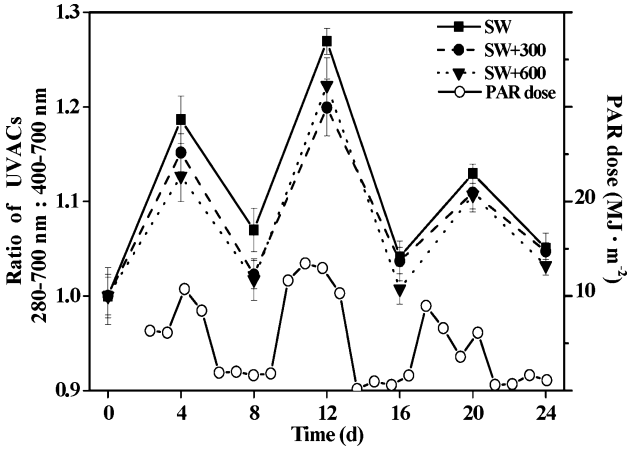

FIG. 8. Ratio of UVAC absorptivity under PAB to those under PAR treatments in the thalli grown at different nitrate concentrations when they were switched back and forth from outdoor to indoor conditions from 1 to 24 August 2005. Data are the means ± SD ($n = 3$). SW, seawater; SW + 300, seawater enriched with 300 μm nitrate; SW + 600, seawater enriched with 600 μm nitrate; UVAC, UV-absorbing compound.

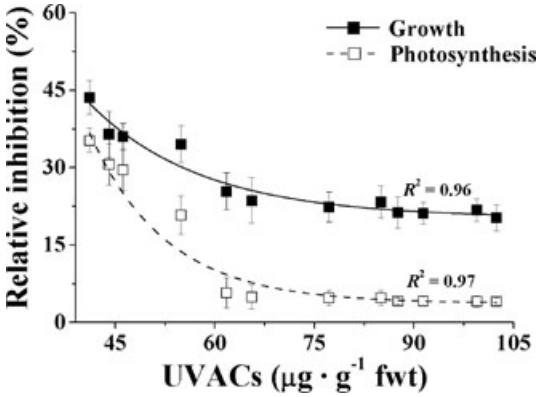

FIG. 9. UVR-induced inhibition (%) on growth and photosynthesis as a function of UVAC absorptivity. Data are the means ± SD ($n = 3$). UVAC, UV-absorbing compound.

attributed to nitrate reduction, the contribution of which is usually <10%. On the other hand, when different radiation treatments with or without UVR were compared at the same nitrate level, any effect of nitrate reduction could be ruled out.

The relative inhibition of the Pn caused by UVR was as low as 4% at 102 μg UVACs · g^{-1} (fwt) but was about 35% at 41 μg UVACs · g^{-1} (fwt). The 4% inhibition caused by UVR under the NO_3^--enriched conditions reflected a chronic inhibition of the photosynthetic apparatus or key enzymes involved in photosynthetic production (Bischof et al. 2000, 2002). Since the presence of UVA resulted in an insignificant effect on the net photosynthesis of *G. lemaneiformis* (Fig. 5), the chronic inhibition was caused by UVB. UVACs, such as MAAs, particularly in red algae (Karsten et al. 1998), are known to play protective roles against both UVA and UVB (Oren and Gunde-Cimerman 2007). Although the composition of UVACs was not identified with HPLC, absorption spectra of all the extracts exhibited a major peak at 325 nm (Fig. 6), indicating a specific UV-absorption feature represented by MAAs. The constant absorption peak and its altered absorptivity demonstrated a consistent response of MAAs to UVR. Accumulation of the UVACs provided more protection in photosynthesis than in growth by shielding key molecules from being damaged. UVR-related inhibition of growth was about 33% higher in SW and about 400% higher in SW + 300 or SW + 600 than inhibition of photosynthesis. Such a discrepancy reflects the nonproportional inhibition of UVR on growth and photosynthesis. UVR (UVA and/or UVB) may have stimulated the dark respiration (Aguilera et al. 1999), and enhanced respiration can result in loss of biomass and subsequent lower growth rate. On the other hand, solar UVB may also affect the cell division due to its damage to DNA (Pakker et al. 2000b, Buma et al. 2001, Roleda et al. 2006a). Recently, UVR was reported

to affect the transverse division of cells in *Porphyra haitanensis* juveniles (Jiang et al. 2007).

Mycosporine-like amino acids are water-soluble substances with nitrogen substituent of an amino acid or its imino alcohol (Sinha et al. 2001). Although they are known to play a protective role against UVR (Oren and Gunde-Cimerman 2007), little is known about their localization in the cells (Pérez-Rodríguez et al. 2003). Since their accumulation provided more sufficient protection of photosynthesis than growth against UVR, the MAAs might be sheltering or surrounding the photosynthetic machinery in *G. lemaneiformis*, On the other hand, the synthesis of UVACs must be accomplished at the cost of growth as indicated in the green macroalga *Ulva pertusa* (Han and Han 2005). The ratio of the UVAC absorptivity under PAR + UVR to PAR alone treatments was higher under the nitrate-limited condition, while the growth rate was higher in the absence of UVR (PAR alone) (Figs. 3 and 8). This finding implies that that the alga used nitrogen for the synthesis of UVACs at the cost of growth, reflecting metabolic balance between growth and the protective mechanism. Nitrate absorbed by algae is allocated to different pathways; the uptake and metabolism of nitrogen has been reported to be inhibited by UVR (Döhler 1992, 1994, Braune and Döhler 1994, 1996, Rai and Rai 1997, Huovinen et al. 2007). Synthesis of UVACs can compete with that of proteins for nitrogen, which is critical for an alga to survive N-limited and UVR-damaging conditions. Consequently, UVR-induced inhibition of growth and photosynthetic production may change in accord with changes in the chemical and physical environments in coastal ecosystems.

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