

Solar UV Radiation Drives CO₂ Fixation in Marine Phytoplankton: A Double-Edged Sword¹

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Photosynthesis by phytoplankton cells in aquatic environments contributes to more than 40% of the global primary production (Behrenfeld et al., 2006). Within the euphotic zone (down to 1% of surface photosynthetically active radiation [PAR]), cells are exposed not only to PAR (400–700 nm) but also to UV radiation (UVR; 280–400 nm) that can penetrate to considerable depths (Hargreaves, 2003). In contrast to PAR, which is energizing to photosynthesis, UVR is usually regarded as a stressor (Häder, 2003) and suggested to affect CO₂-concentrating mechanisms in phytoplankton (Beardall et al., 2002). Solar UVR is known to reduce photosynthetic rates (Stemann Nielsen, 1964; Helbling et al., 2003), and damage cellular components such as D1 proteins (Sass et al., 1997) and DNA molecules (Buma et al., 2003). It can also decrease the growth (Villafañe et al., 2003) and alter the rate of nutrient uptake (Fauchot et al., 2000) and the fatty acid composition (Goes et al., 1994) of phytoplankton. Recently, it has been found that natural levels of UVR can alter the morphology of the cyanobacterium *Arthrospira* (*Spirulina*) *platensis* (Wu et al., 2005b).

On the other hand, positive effects of UVR, especially of UV-A (315–400 nm), have also been reported. UV-A enhances carbon fixation of phytoplankton under reduced (Nilawati et al., 1997; Barbieri et al., 2002) or fast-fluctuating (Helbling et al., 2003) solar irradiance and allows photorepair of UV-B-induced DNA damage (Buma et al., 2003). Furthermore, the presence of UV-A resulted in higher biomass production of *A. platensis* as compared to that under PAR alone (Wu et al., 2005a). Energy of UVR absorbed by the diatom

Pseudo-nitzschia multiseriis was found to cause fluorescence (Orellana et al., 2004). In addition, fluorescent pigments in corals and their algal symbiont are known to absorb UVR and play positive roles for the symbiotic photosynthesis and photoprotection (Schlichter et al., 1986; Salih et al., 2000). However, despite the positive effects that solar UVR may have on aquatic photosynthetic organisms, there is no direct evidence to what extent and how UVR per se is utilized by phytoplankton. In addition, estimations of aquatic biological production have been carried out in incubations considering only PAR (i.e. using UV-opaque vials made of glass or polycarbonate; Donk et al., 2001) without UVR being considered (Hein and Sand-Jensen, 1997; Schippers and Lürling, 2004). Here, we have found that UVR can act as an additional source of energy for photosynthesis in tropical marine phytoplankton, though it occasionally causes photoinhibition at high PAR levels. While UVR is usually thought of as damaging, our results indicate that UVR can enhance primary production of phytoplankton. Therefore, oceanic carbon fixation estimates may be underestimated by a large percentage if UVR is not taken into account.

DIRECT AND INDIRECT EVIDENCE OF UV-DRIVEN CARBON FIXATION

There was a significant photosynthetic carbon fixation (Fig. 1A) when surface phytoplankton assemblages (collected during summer 2005 and summer 2006) were exposed to solar UVR alone (i.e. when PAR was filtered out). The rate of carbon fixation per unit chlorophyll (Chl) was significant ($P < 0.05$) even under low levels of UVR (i.e. $< 5 \text{ W m}^{-2}$). UVR-energized CO₂ fixation increased linearly with increasing UVR, and it was not saturated at 33.3 W m^{-2} (i.e. about half of the noontime irradiance, about $100 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$). The apparent UVR utilization efficiency (i.e. the initial slope of the carbon fixation versus UVR relationship) was about 8×10^{-3} and $6 \times 10^{-3} \mu\text{g C} (\mu\text{g Chl a})^{-1} \text{ h}^{-1} (\mu\text{mol photons m}^{-2} \text{ s}^{-1})^{-1}$ for UV-A and UVR, respectively. The difference in the rate of carbon fixation between samples receiving UV-A + UV-B or

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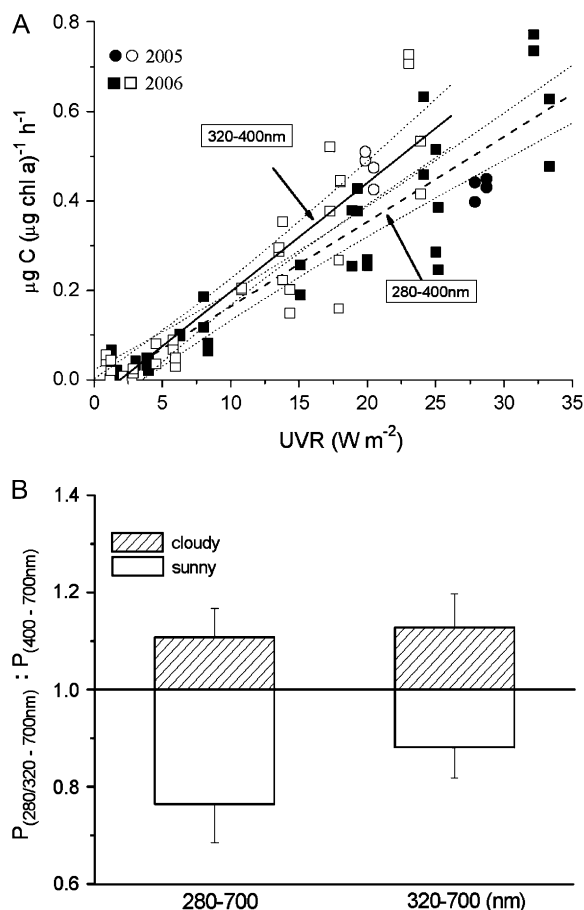


Figure 1. A, CO_2 -fixation rates measured in phytoplankton assemblages as a function of solar UVR (280–400 nm, black symbols) or UV-A (320–400 nm, white symbols) on August 4, 2005, September 27, 2005, and July 8 to 10, 2006. The solid and dashed lines represent a linear fit of the data ($P < 0.0001$), while the dotted lines are the 95% confidence limit. Mean solar irradiances ranged from 312.0 to 486.5, 44.6 to 59.2, and 1.97 to 2.56 W m^{-2} for PAR, UV-A, and UV-B, respectively, throughout the incubations. B, CO_2 -fixation rates of phytoplankton assemblages exposed to PAR + UV-A + UV-B (280–700 nm) and PAR + UV-A (320–700 nm) as compared to those exposed only to PAR. The mean irradiances of PAR, UV-A, and UV-B during the incubations were 224.3, 37.2, and 1.76 W m^{-2} on the cloudy days (July 29, September 10, and September 22, 2005), and 318.3, 50.1, and 2.33 W m^{-2} on the sunny days (August 4 and September 27, 2005). The mean photosynthetic carbon fixation rates under PAR alone were 7.16 and 4.98 $\mu\text{g C } (\mu\text{g Chl a})^{-1} \text{ h}^{-1}$ on cloudy and sunny days, respectively. The vertical bars represent SD ($n = 4$ to approximately 6).

merely UV-A was only significant at UVR levels $>13.3 \text{ W m}^{-2}$, with samples receiving UV-B reducing photosynthesis up to 20% ($P < 0.05$) at the highest radiation tested (i.e. approximately 25.0 W m^{-2}).

The photosynthetic carbon fixation of these phytoplankton assemblages, at the irradiance received at the surface of the ocean, was compared under different weather conditions (i.e. cloudy and sunny days). On cloudy days, UVR + PAR resulted in a significant enhancement of photosynthetic rates, as compared to PAR alone, with a maximum increase of 13% due to UV-A

(Fig. 1B, dashed bars) and a decrease of 2% due to UV-B. On sunny days, however, there was a significant decrease in photosynthetic rates by as much as 24% due to UVR (Fig. 1B, white bars), with UV-A and UV-B contributing equally to the observed inhibition.

ENHANCEMENT AND INHIBITION OF PRIMARY PRODUCTION IN THE WATER COLUMN

Photosynthesis versus irradiance (P - E) curves were obtained for these phytoplankton assemblages under solar radiation with or without UVR (Fig. 2A); positive and negative impacts of UVR were evidenced similarly to those determined during cloudy and sunny days. The apparent light utilization efficiency was 0.078 and 0.046 $\mu\text{g C } (\mu\text{g Chl a})^{-1} \text{ h}^{-1} (\mu\text{mol photons m}^{-2} \text{ s}^{-1})^{-1}$ for samples receiving PAR + UVR or PAR alone, respectively. The maximum photosynthetic rate, however, was approximately 20% lower under UVR + PAR [7.13 $\mu\text{g C } (\mu\text{g Chl a})^{-1} \text{ h}^{-1}$] than under PAR alone [8.90 $\mu\text{g C } (\mu\text{g Chl a})^{-1} \text{ h}^{-1}$]. The photosynthetic carbon fixation became saturated at PAR irradiances of 135 and 275 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ for samples receiving PAR + UVR or only PAR, respectively. At PAR irradiances lower than 300 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$, there was a significant utilization of UVR ($P < 0.05$), and phytoplankton assemblages had a higher rate of photosynthesis than when samples were exposed only to PAR. On the other hand, at PAR irradiances higher than 680 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$, a significant UVR-induced photosynthetic inhibition ($P < 0.05$) was observed and increased with increasing irradiance.

During sunny days the irradiance levels at depths deeper than 1.7 m were such that photosynthesis was not saturated, and samples receiving UVR had higher daytime carbon fixation than those exposed only to PAR (Fig. 2B). UVR inhibited the daily photosynthetic production by as much as 25% at the surface. On the other hand, during cloudy days the whole water column was below the saturating light level determined from P versus E curves, and, thus, the carbon fixation was higher in the UVR + PAR treatment (white symbols in Fig. 2B) at all depths. The daily primary production integrated for the euphotic zone based on the depth distribution of carbon fixation (Fig. 2B) ranged from 136 to 565 mg C m^{-2} for samples exposed to full solar radiation and from 93 to 528 mg C m^{-2} for samples exposed only to PAR. For all the data points obtained during the summer period, we compared the ratio of integrated production of samples receiving full solar radiation to that of samples receiving only PAR as a function of the daily PAR dose (Fig. 2C). Enhanced photosynthetic efficiency by UVR resulted in up to 46% higher daily primary production under reduced levels of solar radiation (Fig. 2, B and C). Even under the brightest weather conditions, solar UVR could still raise daily primary production by 7% (Fig. 2C). For cloudy days, when photosynthetic carbon fixation was enhanced with UVR (Fig. 1C), daily PAR doses of

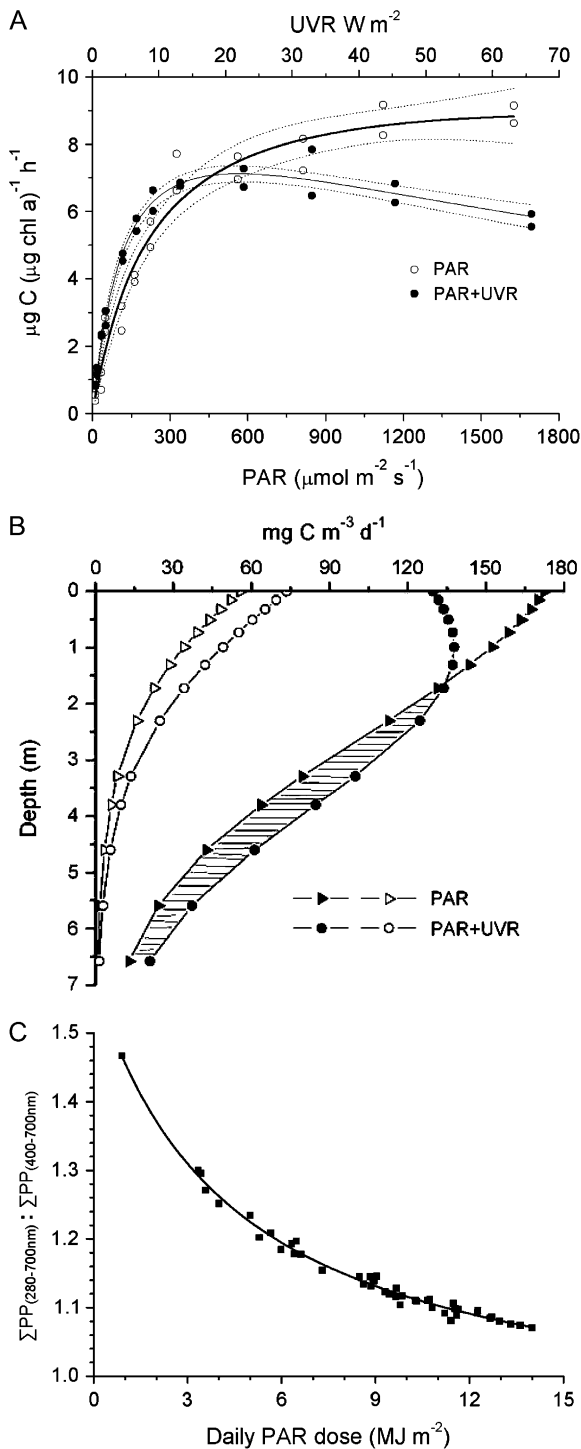


Figure 2. A, Photosynthesis versus irradiance curve under PAR (white circles) and PAR + UVR (black circles) conditions; dotted lines represent 95% confident limit. The mean irradiances (PAR, UV-A, and UV-B) during the incubations (August 6 and 8, 2005) were 321, 50.5, and 2.1 W m^{-2} , respectively. B, Vertical distribution of estimated daily photosynthetic production on a sunny day (black symbols; July 5, 2006) and a cloudy (white symbols; August 13, 2006) day. The doses (and mean irradiances) of PAR for these days were 14 (280 W m^{-2}) and 0.9 MJ m^{-2} (19.6 W m^{-2}), respectively. Note that even on the sunny day without cloud coverage, UVR-enhanced production (shaded areas) is

4.8 MJ m^{-2} led to approximately 20% higher daily primary production when both PAR and UVR were available for the euphotic zone.

PHYTOPLANKTON COMPOSITION AND UV-ABSORBING COMPOUNDS

During the study period, microplankton cells ($>20 \mu\text{m}$) accounted for 18% and 38% of total Chl a concentration (that ranged from 1.12 to approximately 7.79 and 6.79 to approximately 8.50 $\mu\text{g L}^{-1}$ during the summers of 2005 and 2006, respectively). The microplankton species were mainly represented by the diatom genera *Chaetoceros*, *Rhizosolenia*, *Pseudonitzschia*, and *Skeletonema*, whereas picnanoplankton ($<20 \mu\text{m}$) was represented by unidentified monads and flagellates. Absorption of the methanolic extracts of the phytoplankton assemblages showed a distinguishable absorption peak between 330 to 340 nm, indicating the presence of UV-absorbing compounds (Fig. 3).

DATA EVALUATION, POSSIBLE MECHANISMS INVOLVED, AND IMPLICATION

Our results demonstrate that solar UV-A can be used for CO_2 fixation by tropical marine phytoplankton assemblages as an additional source of energy for photosynthesis, though it occasionally causes photoinhibition in the presence of high PAR levels. UVR-driven carbon fixation would be higher under natural levels of solar radiation since photosynthetic carbon fixation rate was not saturated under approximately half (33.3 W m^{-2} , maximum level that the UG11 filter allows) of the incoming noontime UVR (Fig. 1A). However, in the presence of PAR, UVR-induced photoinhibition was significant ($P < 0.05$) at levels $>20 \text{ W m}^{-2}$ (corresponding PAR, 680 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ or 147 W m^{-2} ; Fig. 2A). UVR is known to damage the D1 protein of PSII and to inhibit photosynthesis (Sass et al., 1997). The energy of UVR, which brought about the photosynthetic carbon fixation in the absence of PAR (Fig. 1A), might be transferred through a pathway different from that of PAR (i.e. not via PSII) so it could simultaneously inhibit PAR-related photosynthesis and drive carbon fixation. The measured rate in the presence of PAR is the balanced value between inhibition and enhancement of carbon fixation. When UVR-enhanced photosynthetic carbon fixation was compared between sunny and cloudy days (Fig. 1B), the mean PAR irradiance during the measurements on the cloudy day was 1,030 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ (224.3 W m^{-2}), much higher than 680 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ in

larger than the UVR-related reduction (open area enclosed by the lines). C, Daily primary production (ΣPP) ratios of samples exposed to the full solar spectrum compared to those exposed to PAR only. The estimation of ΣPP was based on the P versus E curves. The relationship of solar daily dose with ΣPP ratio is significant ($P < 0.001$; $y = 0.91 \times \exp[3.19/(x + 5.82)]$); $R^2 = 0.99$.

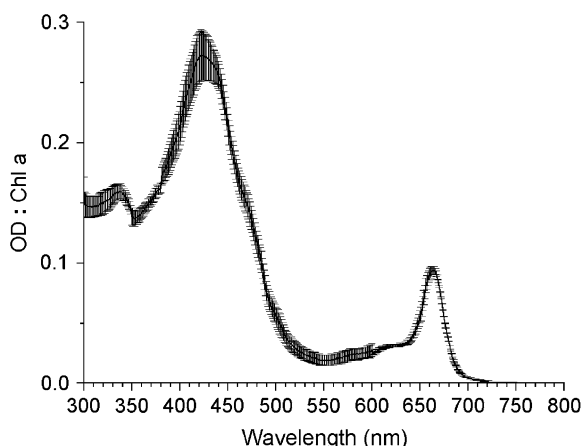


Figure 3. Optical density of methanol extracts from the natural phytoplankton assemblages (July 8–10, 2006). Vertical bars represent SD ($n = 3$).

the P versus E curve (Fig. 2A), at which photosynthesis became inhibited. This apparent discrepancy seems to be related to the fluctuating patterns of the solar radiation. During the incubation on the cloudy day (Fig. 1B), the irradiance of PAR ranged from 373 to 1,881 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (0.3 times per minute with irradiance fluctuations $>15\%$), whereas, during P versus E curve measurements on the sunny day (Fig. 2A), no discernible fluctuation of solar radiation was observed. Fluctuating solar radiation can affect the balance between inhibition and enhancement of photosynthetic carbon fixation due to reduced damage and enhanced repair at frequently reduced levels of sunlight. PAR-related (Marra, 1978) or UVR-related (Helbling et al., 2003) inhibition of phytoplankton photosynthesis was modulated by fluctuating PAR or solar radiation. Therefore, different overcast conditions, with varied extent of solar radiation fluctuations due to cloud movements, are the key to determine the extent of UVR enhancement and the boundary irradiance at which UVR-induced inhibition exceeds enhancement. On the other hand, the apparent photosynthetic efficiency (α) for the P versus PAR curve (Fig. 2A) was about 7.7 (5.8) times that for the P versus UVR (UV-A) curves (Fig. 1A). The lower α values for UVR reflect its lower efficiency for the carbon fixation. Since pigments and other cellular components absorb UVR and PAR to different extents, they can result in difference in transmission and use efficiencies between them. The α value of P versus PAR + UVR curve (Fig. 2A) was about 50% higher than the sum of UVR-related (Fig. 1A) and PAR-related α values. This could be related to the interactive effects of PAR and UVR that might not be seen under the respective treatment. Different phytoplankton assemblages collected on different days might also have accounted for the observed difference. Mechanistic thinking about these responses can be directed to: (1) UV-absorbing compounds (mainly, mycosporine-like amino acids [MAAs]) might function as antenna

compounds to transfer energy (in addition to their role as protective sunscreens); and (2) enzymes capable for carboxylation or CO_2 -acquisition processes might be UV-sensitive and stimulated by UVR at low irradiance levels.

UVR can be absorbed by a number of cellular substances, such as proteins and ATP (Kondo et al., 1979). Even Chl a has a partial absorption of the near UV range (Harris and Zscheile, 1943). However, the dominant UV-absorbing compounds found in phytoplankton cells are MAAs (absorption range 310–360 nm) in eukaryotic cells (Dunlap et al., 1995) and scytonemin (absorption peak at 370 nm) in prokaryotic species (Garcia-Pichel et al., 1992). An oligosaccharide-mycosporine amino acid with absorption peaks of 312 nm and 335 nm was also reported in a cyanobacterium (Böhm et al., 1995). MAAs are known to play an important role as a cellular screen to filter UVR, limiting its harmful effects (Dunlap et al., 1995). Mycosporine-like Gly, shinorine, and mycosporine-Gly/Val were found to be able to transmit absorbed UVR energy to Chl a in the haptophyte *Phaeocystis antarctica* (Moisan and Mitchell, 2001). In the diatom *P. multiseriis*, absorbed UVR energy was also evidenced to generate Chl fluorescence (Orellana et al., 2004). The UV energy absorbed by the corals and their algal symbiont was found to emit fluorescence (Schlichter et al., 1986; Salih et al., 2000). It is thus possible that quanta in the UVR region that caused photosynthetic carbon fixation by the phytoplankton assemblage (Fig. 1A) could be absorbed by MAAs (Fig. 3) and then transferred to Chl a ; the transferred energy is utilized to drive the photosynthetic carbon fixation (Figs. 1, A and B, and 2A). In fact, theoretically, and from an evolutionary point of view, phytoplankton cells may have devised the use of short wavelengths down to 300 nm for photosynthesis (Neori et al., 1988; Holm-Hansen et al., 1993).

UV-A and blue light are known to signal photoreponses via two types of photoreceptors, cryptochromes and phototropins (Brunner et al., 2000; Lin, 2002; Huang et al., 2004). Previous studies have shown that low levels of UVR can enhance CO_2 acquisition in the diatom *Skeletonema costatum* by stimulating the extracellular (periplasmic) carbonic anhydrase (data not shown). Blue light was demonstrated to increase the activity of plasma membrane H^+ -ATPase in a brown alga, *Laminaria digitata* (Klenell et al., 2002). UV-A, as a neighboring radiation of blue light, may serve a similar function and accelerate CO_2 acquisition and fixation. Intracellular inorganic carbon concentration increased, though photosynthetic carbon fixation was inhibited, in the marine green alga *Dunaliella tertiolecta* when exposed to UV-B (Beardall et al., 2002). CO_2 -concentrating mechanisms (Giordano et al., 2005) in phytoplankton may be affected by UVR.

Phytoplankton cells, circulating up and down by waves or mixing in the ocean, are frequently exposed to reduced levels of solar radiation even at noontime; thus, they tend to be less photoinhibited by high levels of UVR and PAR (due to short exposures) than

previously suggested. Because attenuations of PAR and UVR are not the same in any marine habitat so far examined, effects of UVR on primary production could differ between oceanic and coastal waters. Nevertheless, total oceanic primary production in tropical areas could have been previously underestimated for the euphotic zone. Since UVR may affect the new (potentially sedimentable) productivity of the ocean via influencing phytoplankton photosynthesis, the marine biological removal of dissolved inorganic carbon with UVR being considered would add to the oceanic sink of CO₂ approximated to date (Sabine et al., 2004). Taking into account our data for the summers of 2005 and 2006, we calculated that the daily primary production for the tropical coastal euphotic zone would be underestimated by as much as 13% if solar UVR is not considered. This proportion of “unaccounted” CO₂ fixation would be higher in seasons with more cloudy days.

MATERIALS AND METHODS

Study Area and Sampling

This study was performed in a coastal area of the South China Sea (23°29' N, 117°06' E) during the summers of 2005 and 2006. Surface seawater samples were collected 500 m offshore with a 10-L acid-cleaned (1 N HCl) carboy in the morning and returned to the laboratory (within 15 min) of the Marine Biological Station of Shantou University, where the experiments were carried out as described below.

Solar Radiation Treatments and Monitoring

To determine UVR effects upon phytoplankton assemblages, solar radiation treatments were implemented (duplicate or triplicate) as follows: (1) PAR + UV-A + UV-B uncovered quartz tubes; (2) PAR + UV-A quartz tubes covered with Folex 320 filter (to filter out UV-B); (3) PAR-alone quartz tubes covered with Ultraphan 395 filter (to filter out UVR); (4) UV-A + UV-B quartz tubes covered with UG11 filter (to filter out PAR); (5) UV-A quartz tubes covered with UG11 + Folex 320 filter (to filter out PAR and UV-B); and (6) darkness quartz tubes covered with UG11 filter and Ultraphan 395 (control A) or covered with aluminum foil (control B).

The transmission spectra of the filters Folex 320 (Montagefolie; no. 10155099; Folex) and Ultraphan 395 (UV Opak; Digefra) are given by Figueroa et al. (1997). UG11 filter (Schott) cuts off 100% PAR and transmits 53.7% of UV-A and 63.8% of UV-B (when measured at noontime under sunlight). The uncovered quartz containers received 4% higher PAR radiation in water (measured by inserting a PAR sensor inside the quartz container) compared with the covered tubes with 395 or 320 filters due to the reflection caused by these filters. This was calibrated for establishment of the photosynthesis versus irradiance (*P-E*) relationship.

To determine the UVR-only impacts on carbon fixation, quartz tubes containing surface seawater were placed in a PAR-opaque box with UG11 filter sandwiched and sealed in the cover. Thus, the *P-E* curves were obtained in the absence of PAR under no and up to five layers of neutral density screens so that UVR irradiance varied from 53.7 to <1.6%. To calculate the apparent utilization efficiency of UVR, UVR irradiance was converted from W m⁻² to photon flux by multiplying by 3.02 according to Neale et al. (2001) and solar spectrum estimated using the STAR software (Ruggaber et al., 1994). To determine the impacts of PAR with and without UVR, measurements of the photosynthetic carbon fixation were carried out in different weather conditions (i.e. cloudy and sunny) under the radiation treatments as described above.

Incident solar radiation (UV-B: 280–315 nm; UV-A: 315–400 nm; PAR: 400–700 nm) was continuously monitored using a broadband solar radiometer (ELDONET; Real Time Computer). This instrument measures every second direct and indirect radiation (Ulbrich integrating sphere) and records the averaged data at 1-min intervals (Häder et al., 1999).

Determination of Photosynthetic Carbon Fixation and Estimation of Primary Production

Photosynthetic carbon-fixation rates were determined as follows: Water samples, pre-filtered by a 180- μ m-pore mesh (to eliminate large zooplankton specimens) were dispensed into 20-mL quartz tubes and inoculated with 0.1 mL of 5 μ Ci (0.185 MBq) of labeled sodium bicarbonate (Amersham). Then the samples were incubated for 3 h centered on local noon to determine photosynthetic rates in a water bath with running surface seawater to control temperature (27°C–30°C). After incubation, samples were filtered onto Whatman GF/F glass fiber filters (25 mm), and filters were placed into 20-mL scintillation vials, exposed to HCl fumes overnight, and dried (45°C). Scintillating cocktail (PerkinElmer) were added to the filters and the incorporated ¹⁴C counted using a liquid scintillation counter (LS 6500; Beckman Coulter; Holm-Hansen and Helbling, 1995).

P versus *E* curves, under PAR + UVR and PAR only, were obtained on August 6 and 8, 2005, under six light levels. The data for the curves were fitted according to the model of Eilers and Petters (1988) as:

$$P = E/(aE^2 + bE + c),$$

where *P* is the production [μ g C (μ g Chl a)⁻¹ h⁻¹], *E* is the irradiance (μ mol m⁻² s⁻¹), and *a*, *b*, and *c* are the adjustment parameters. Since the uncovered quartz containers received 4% higher PAR than those covered with the cutoff filters either under low or high levels of solar radiation and this higher portion of PAR might affect the comparison of irradiance-limiting photosynthetic rate with or without UVR, the PAR values were calibrated by multiplying by 0.96 for the *P-E* curve with Ultraphan 395 filter. The daily primary production (Σ PP) was estimated over the period (July 1–September 30, 2005) according to the *P-E* curves and time- and depth-integrated models (Behrenfeld and Falkowski, 1997), assuming constant PAR attenuation coefficient *K_d* of 0.7 m⁻¹ (on August 4, 2006), vertical Chl a distribution (mean of 1.6 μ g L⁻¹), and photosynthetic characteristics. The daily photosynthetic production was integrated per minute according to the irradiance values and the *P-E* curves, and this was then integrated per 10% of PAR every depth interval (at the depths <10% surface PAR, 7%, 4%, 2%, and 1% were employed). The daily photosynthetic production was subsequently integrated for the euphotic zone as follows:

$$\sum PP = \int_{t=\text{sunrise}}^{\text{sunset}} \int_{Z=0}^{z_{eu}} PAR(t, z)/(a \times PAR^2(t, z) + b \times PAR(t, z) + c),$$

where *a*, *b*, and *c* are the adjustment parameters in the *P-E* curve.

Measurements of Pigments

At the beginning of experiments, samples were taken to determine absorption of methanolic extract, Chl a concentration, and species composition. Two liters of seawater were filtered onto a Whatman GF/F glass fiber filter (47 mm), and then the filtrate was extracted with absolute methanol for 3 h at room temperature. The extract was subsequently determined for the optical density using a scanning spectrophotometer (UV 2501-PC; Shimadzu). Chl a concentration was calculated according to Porra (2002). To determine Chl a in the picoplankton fraction, a subsample was prefiltered through a Nitex mesh (20 μ m) and the extraction of photosynthetic pigments was done as described above. The quantity and quality analysis of phytoplankton cells fixed with buffered formalin (final concentration of 0.4% in the sample) was carried out using an inverted microscope (Olympus IX51) after settling 10 mL of sample for 24 h (Villafañe and Reid, 1995).

Data Analysis

The Kruskal-Wallis nonparametric test was used to determine significant differences between the estimated parameters (confidence level = 0.05); the correlation between variables were established using a Kendall's τ test.

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