Variation in UV irradiance related to stratospheric ozone levels affects photosynthetic carbon fixation of winter phytoplankton assemblages from surface coastal water of the South China Sea

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Abstract
Solar ultraviolet radiation (UVR, 280–400 nm) in aquatic environments significantly affects photosynthetic carbon fixation by marine phytoplankton. To document such UV-related effects in Chinese waters, we measured in-situ photosynthetic carbon fixation in the coastal surface seawater of the South China Sea under solar radiation in the presence and in the absence of UVR during the winter monsoon period (15 October to 16 December 2005). Phytoplankton biomass (Chl a) ranged from 1.40 to 3.79 µg Chl a l⁻¹, 75–98% of which was accounted for by piconanoplankton cells (<20 µm). The photosynthetic carbon fixation obtained under photosynthetically active radiation (PAR) at noon varied from 5.0 to 37.8 µg C l⁻¹ h⁻¹, whereas the carbon fixation rate ranged from 2.36 to 9.98 µg C (µg Chl a)⁻¹ h⁻¹. UV-A and UV-B significantly lowered the photosynthetic rates by up to 38% and 29%, respectively. Reduced levels of UV-A on cloudy days resulted in enhanced photosynthetic carbon fixation by up to 6.6%. The UV-A-induced inhibition of photosynthetic carbon fixation by phytoplankton assemblages in the coastal water was negatively correlated with the temperature levels but positively related to pH values. Stratospheric ozone variation affected the UV-B/PAR ratio, which then influenced the UV-B-related inhibition of phytoplankton photosynthesis: a higher UV-B/PAR ratio led to higher UV-B-related inhibition. Changes in salinity, PAR and cell-fractions (>20 or <20 µm) insignificantly interacted to influence the UV-induced inhibition of carbon fixation.

Key words: Carbon fixation, phytoplankton, temperature, UVR, South China Sea

Introduction
Marine phytoplankton species play important roles as primary producers, supporting food chains in marine ecosystems, and as a driving force for the marine biological CO₂ pump to absorb atmospheric CO₂ into the oceans (Saino et al. 2004; Wu et al. 2008). Their photosynthesis is driven by photosynthetically active radiation (PAR, 400–700 nm) in the euphotic zone (1% of surface visible radiation). At the same time, they are also exposed to ultraviolet radiation (UVR, 280–400 nm), the biologically effective levels of which can penetrate up to 60 m in open oceans (Tedetti & Sempé 2006). Solar UVR is known to reduce growth and photosynthetic rate (Gao et al. 2008; Li et al. 2009, 2011), bleach pigments (Worrest et al. 1978), damage DNA molecules (Buma et al. 2003; Gao et al. 2008), leading to cell death (Agusti & Llabrés 2007), and so ultimately to change the species composition (Marcoval et al. 2008) and to influence primary production (Zhou et al. 2009; Wu et al. 2010) and food chains (Häder et al. 2007). On the other hand, moderate levels of UV-A (315–400 nm) are found to simulate the repair of UV-B (280–315 nm)-damaged DNA (Karentz et al. 1991; Buma et al. 2003) and coastal phytoplankton photosynthetic carbon fixation (Barbieri et al. 2002; Helbling et al. 2003; Gao et al. 2007a, b).

Generally, the diversity of phytoplankton species changes in response to environmental changes associated with seasonal shifts, leading to inter-seasonal
succession in species composition (Liu et al. 2002). Photophysiological responses of phytoplankton cells to solar UV usually differ according to species and to environmental conditions (Häder et al. 2007). Reduced availability of nutrients leads to increased sensitivity in the dinoflagellates Akashiwo sanguinea and Gyrodinium instriatum to UV (Litchman et al. 2002); and increased temperature reduces UV-induced damage to the cyanobacterium Arthrospira (Spirulina) platensis (Marcoval et al. 2008) and to the diatoms Chaetoceros neogracile and Thalassiosira weissflogii (Halac et al. 2010). Fast mixing decreases UV-induced inhibition of photosynthetic carbon fixation (Helbling et al. 2003; Zhou et al. 2009). In the South China Sea (SCS), high water temperature and shallow stratification (even in coastal waters) prevails in summer (Ning et al. 2004; Li et al. 2007a, 2012); while in winter, the influence of the northeast monsoon leads to intensive mixing, which often triggers blooms of phytoplankton due to enrichment by nutrients pumped from the deeper layers (Chen et al. 2006). Mixing caused by the monsoons can decrease the temperature of surface waters sharply by accelerating the heat dissipation or/and water exchanges between surface and deep layers (Chu et al. 1997). The drastic decrease in temperature can affect all biochemical reactions catalysed by enzymes in phytoplankton cells (Gillooly et al. 2001) and thus may alter their responses to UV radiation. However, little has been documented concerning the effects of solar UV on natural phytoplankton assemblages during winter.

The SCS is the second largest marginal sea with an area of $3.5 \times 10^6$ km$^2$. Most of the previous studies on primary production of the SCS coasts have been carried out in summer (Helbling et al. 2003; Gao et al. 2007a,b; Li et al. 2009, 2011); winter period data are essential to look at UV effects during different seasons, as temperature and nutrient loads are largely different during different seasons. A scarcity of winter data makes it difficult to estimate the impacts of solar UV radiation on the primary productivity of this area on an annual basis (Wu et al. 2010). The aim of this article was to examine the impacts of solar UV on the photosynthetic carbon fixation of winter phytoplankton assemblages from a coastal area of the SCS.

Materials and methods

Study area/sampling protocol

From 15 October to 16 December 2005 (Julian days 287–350), we carried out studies on the natural phytoplankton assemblages from the coastal waters (10 m deep, 500 m off the coast) of Nan’ao Island (23°24’N, 117°07’E) in the SCS (Figure 1). In the morning, around 9:00 h, surface seawater was collected with an acid-cleaned (1N HCl) polycarbonate carboy, immediately after measuring the vertical profiles of temperature and salinity. The collected water samples were transported to the laboratory within 15 min, and the following experiments and analyses were performed.

Measurement of solar radiation and other factors

Incident solar radiation was monitored continuously with a broadband radiometer (ELDONET, Real Time Computers Inc., Germany) placed on the roof of the Marine Biological Station (23°24’N, 117°07’E; 30 m away from the incubation site) of Shantou University. This device monitors three wavebands, UV-B (280–315 nm), UV-A (315–400 nm) and PAR (400–700 nm) at the same time and records the minute-averaged values (Häder et al. 1999). A submersible version of this device with the same channels as above, as well as temperature and pressure sensors, was used to measure the underwater radiation. The cut-off filters reduced 4% of the PAR (Gao et al. 2007b) in the water owing to reflection; and the measured UV-A was approximately a 5-nm waveband less than the exposed one. Therefore, the cells received about 4% less PAR and about 2% less UV-A, compared to the measured irradiance or to the unwrapped quartz tubes.

Profiles of salinity and temperature in the water column of the sampling area were determined with a CTD (YSI 600XL, Yellow Springs Instruments, USA) at intervals of 20 cm. The pH value in surface water was measured using an Oakton pH meter, which was regularly calibrated using standard N.B.S. (National Bureau of Standards) buffer.
Experiments

Surface seawater, pre-filtered through a 180 μm pore mesh (to remove large zooplankton), was dispensed into 30 ml quartz tubes, and inoculated with NaH¹⁴CO₃ solution to determine carbon fixation by the phytoplankton (see below); and three radiation treatments were employed (duplicates for each) to examine the UV-induced impacts as follows:

(a) PAB treatment: unwrapped quartz tubes, samples receiving full sunlight;
(b) PA treatment: quartz tubes covered with Folex UV cut-off filter (Montagefolie, No. 10155099, 50% transmission at 320 nm), samples receiving irradiance above 320 nm;
(c) P treatment: quartz tubes wrapped in Ultra-phran UV Opak Digefra foil (50% transmission at 395nm), samples receiving irradiance above 395 nm.

The transmission spectra of these foils are available elsewhere (Zheng & Gao 2009). In addition, duplicate tubes wrapped in aluminium foil were used as dark controls. All the tubes were maintained beneath the surface (2 cm) of a water tank with running surface seawater to maintain the temperature at the same level as the sea surface temperature (SST) (17–27°C). Exposure to solar radiation lasted for 3 h centred on local noon (10:30–13:30 h). In total, 13 experiments were conducted over the study period.

Measurements of photosynthetic carbon fixation

Pre-filtered (180 μm pore-size) surface seawater samples (30 ml each) were inoculated with 100 μl 5 μCi (0.185 MBq) NaH¹⁴CO₃ solution (ICN Radiochemicals, USA) as the tracer, and incubated under the conditions mentioned above. After incubation, the sample was filtered through a Whatman GF/F glass fibre filter (25 mm), which was then placed into a 20 ml scintillation vial, exposed to HCl fumes overnight and dried (55°C, 6 h) to expel the non-fixed ¹⁴C. The dried filter was digested with a 3 ml scintillation cocktail (Perkin Elmer®) in a vial before the radioactivity was measured using a liquid scintillation counter (LS 6500 Beckman Coulter, USA). The photosynthetic rate was calculated according to the description by Holm-Hansen & Helbling (1995).

Chlorophyll a and species analyses

Chlorophyll a (Chl a) was measured by filtering 300–500 ml of surface seawater through a Whatman GF/F glass fibre filter (25 mm), extracting with 5 ml absolute methanol for 3 h at room temperature in darkness; the extracts were scanned using a spectrophotometer (UV 2501-PC, Shimadzu, Japan). Chl a concentration was calculated according to Porra (2002). To determine the Chl a concentration of the pico-nanoplankton fraction (<20 μm), a sub-sample was pre-filtered through a 20 μm pore-size Nitex® mesh, and Chl a density was determined as described above.

For species analysis, the water samples were fixed with buffered formalin (to a final concentration of 0.4% in the sample), and allowed to settle in a 50 ml cylinder of an Utermöhl Chamber (Hydro-Bios, Kiel, Germany) for 24 h. Qualitative and quantitative analyses were then carried out with an inverted microscope (IX51, OLYMPUS, Japan) after removing the supernatant (Villafañe & Reid 1995).

Statistical analysis

Inhibition of the photosynthetic carbon fixation rate caused by UV exposure was calculated as follows:

\[ \text{Inh} \% = \left( \frac{P_{\text{P}} - P_{\text{PA}}}{P_{\text{P}}} \right) \times 100\% \]

where Inh (%) represents the UV-A or -B inhibition; and \( P_{\text{P}} \), \( P_{\text{PA}} \) and \( P_{\text{PAB}} \) the carbon fixation rates under PAR, PAR + UV-A or PAR + UV-A + B treatments, respectively.

Mean and half ranges were used to present the values in the figures. A paired t-test and one-way ANOVA were used to determine any significant differences between the treatments (significant level at \( p < 0.05 \)). Rank correlations between variables were established using a Kendall’s t test. The ratios of UV-A or -B to PAR were used to reflect relative intensity of UV irrespective of cloud cover.

Results

Total ozone column concentration and daily doses of solar radiation for the study period indicated a slight decline towards the end of this study (Figure 2AB). The ozone column concentration over Shantou (data obtained from http://jwockey.gsfc.nasa.gov/) varied from 271 to 216 Dobson Units, with the maximal and minimal values being observed on days 290 and 314 (Figure 2A), respectively. Incident solar radiation also displayed a high variability, due to the cloud cover (Figure 2B). UV-B daily doses varied from 7.8 to 50 kJ m⁻², while those of UV-A from 1.67 to 0.35 MJ m⁻² and those of PAR from 1.84 to 9.80 MJ m⁻² (Figure 2B). The ratio of UV-B to PAR varied from 0.32% to 0.57% (Figure 2A), and that of UV-A to
PAR from 15.0% to 20.5%. SST, salinity (SSS) and pH levels also varied: i.e. the SST values ranged from 26.5 to 16.3 °C, the SSS from 32.3 to 29.9, and the pH from 7.93 to 8.13 (Figure 2C). The typical profiles obtained on Julian day 312 showed the attenuation of solar irradiance and changes in salinity and temperature with increasing water depth (Figure 3). For the study period, the attenuation coefficients of PAR, UV-A and UV-B were in the ranges of 0.58 /C1 0.83 m /C28 1,31 /C1 2.20 m /C28 1 and 2.01 /C1 4.14 m /C28 1, respectively, with the euphotic zone down to 6 /C1 8 m and 1% of surface UV-A to 3 /C1 3.5 m (1.7 /C1 2.3 m for UV-B).

No significant changes in temperature or salinity (Figure 3B) due to mixing were observed from the surface to the bottom of the water column.

Phytoplankton biomass (Chl a) and photosynthetic carbon fixation are shown in Figure 4. Chl a concentration varied between 1.4 (day 344) and 3.8 µg l−1 (day 314), 75–98% of which was represented by pico-nanoplankton cells (<20 µm) (Figure 4A); and the proportion of pico-nano-cells was negatively correlated with total Chl a biomass (R² = 0.44, p < 0.05). The cell concentration ranged from 200 to 2000 cells ml⁻¹, with the diatoms Coscinodiscus spp. Ehrenberg, 1839 and Pleurosigma normanii Ralfs, 1861 as the most abundant species. Unknown pinnate diatoms were also numerically important, while the dinoflagellates were negligible throughout the study period. The carbon fixation rates under PAR alone ranged from a minimum of 2.1 to a maximum of 36.6 µg C l⁻¹ h⁻¹ (Figure 4B) when based on water volume; and varied from 2.7 to 9.98 µg C (µg Chl a)⁻¹ h⁻¹ when based on the Chl a (Figure 4C). Higher carbon fixation capacity per volume of seawater coincided with the higher Chl a concentration and assimilation number as well as the higher solar radiation. The presence of UVR reduced the carbon fixation markedly (Figure 4B,C). UV-B caused 1.89% (day 321) to 29.4% (day 349) inhibition of photosynthesis; while the UV-A induced inhibition of photosynthesis varied from – 6.6% (day 288) to 37.8% (day 342) (Figure 4D). The negative inhibition on the cloudy day reflected photosynthetic enhancement by UV-A. UV-A-induced reduction in carbon fixation appeared to increase with declining seawater temperature (Figure 2C, 4D). The higher UV-B-induced inhibition of photosynthetic carbon fixation concurred with the higher UV-B/PAR ratio around day 314 (Figure 2A, 4D).

When UV-A- or -B-induced inhibition of photosynthetic carbon fixation was analysed against
different physical and chemical parameters (Table I), the UV-A-related inhibition negatively ($p < 0.01$) correlates with SST, but, together with UV-B-related inhibition, positively ($p < 0.05$) correlates with UV-B/PAR ratio and pH levels. Other factors, such as SSS, ozone, PAR, UV-A/PAR and cell-fractions insignificantly ($p > 0.05$) interacted to influence the UV-induced inhibition of carbon fixation.

### Discussion

In this study, we showed that UV-A-induced inhibition of photosynthetic carbon fixation by phytoplankton assemblages in the coastal water of the SCS was negatively related to the temperature levels but positively related to pH values. Stratospheric ozone variation affected the UV-B/PAR ratios, which then influenced the UV-B-induced inhibition of phytoplankton photosynthesis: a higher UV-B/PAR ratio led to higher UV-B-related inhibition.

Shallow light penetration and intensive vertical mixing prevailed in the coastal water of the SCS in winter and re-suspension of particles or benthic cells must have contributed to the rapid attenuation of solar radiation in the water column (Figure 3). The monsoon-induced physical forcing and subsequently enhanced mixing could have circulated the phytoplankton cells even below the euphotic zone (Figure 3), leading to the reduced light being received by the cells (Helbling et al. 2003; Neale et al. 2003; Zhou et al. 2009). Lower primary productivity in the coastal water was found in winter and spring as compared to summer and autumn (Table II), which could be partially accounted for by the changes in depths and speed of the mixing. Lower water temperature in winter could also account for the lower productivity (Gao et al. 2008). The grazing pressure by zooplankton could be another cause of the low density of phytoplankton cells (Alpine & Cloern 1992); however, in this study, pre-filtration of the seawater should have removed most of the zooplankton and, therefore, grazing would have had little effect on the carbon fixation rates measured.

The phytoplankton assemblages during the winter, as shown in the present study (Figure 4D), were more sensitive to UV exposure (especially UV-A) when compared to their summer counterparts (Table II) (Gao et al. 2007a; Wu et al. 2010), although the winter had much less solar radiation than the summer. Differences between the two seasons in terms of phytoplankton community structure, temperature, mixing and pH values could be responsible for the differences in UV-induced inhibition of photosynthetic carbon fixation. The dominant species may have shifted from UV-tolerant to UV-sensitive ones (Marcoval et al. 2008). Picoplankton cells ($< 20 \mu m$) accounted for most of the phytoplankton biomass in terms of Chl a proportion (Figure 4A). Picoplankton DNA is more easily harmed by UV radiation than microplankton (Helbling et al. 2001). Smaller size fractions of phytoplankton also show higher sensitivity to UV in comparison to larger sizes.

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<tr>
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<th>UV-A$_{inh}$</th>
<th>UV-B$_{inh}$</th>
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<tbody>
<tr>
<td>$r$</td>
<td>$p$</td>
<td>$r$</td>
</tr>
<tr>
<td>SST</td>
<td>$-0.72$</td>
<td>$0.005^*$</td>
</tr>
<tr>
<td>SSS</td>
<td>$0.08$</td>
<td>$0.971$</td>
</tr>
<tr>
<td>pH</td>
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<td>$0.006^*$</td>
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<tr>
<td>Ozone</td>
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</tr>
<tr>
<td>PAR</td>
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<td>$0.973$</td>
</tr>
<tr>
<td>UV-A/PAR</td>
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<td>$0.455$</td>
</tr>
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<td>UV-B/PAR</td>
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<td>$0.013^*$</td>
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<tr>
<td>$&lt;20 \mu m$%</td>
<td>$0.14$</td>
<td>$0.628$</td>
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terms of their photosynthetic performance (Li et al. 2009, 2011). Picoplankton cells are known to be inefficient in accumulating UV-screening compounds that protect them from UV-related damage (Garcia-Pichel 1994). Drastic mixing could lead to the higher UV-inhibition on carbon fixation due to the acclimation of the cells to the lower levels of solar radiation (Li et al. 2009). On the other hand, temperature affects the biochemical reactions which are catalysed by enzymes; therefore, it can affect the efficiency of the repair of UV-damaged molecules and UV-protective processes (Buma et al. 2003; Gao et al. 2008; Halac et al. 2010). This explains the phenomenon that higher UV-induced inhibition of the carbon fixation was significantly associated with lower temperatures (Table I). The pH changes during the study period might also have regulated the UV-induced inhibition on the phytoplankton cells through influencing their electrochemical potential of cell membranes (Giordano et al. 2005), and led to the positive correlation of UV-A inhibition and pH levels (Table I). Finally, low levels of UV and PAR are known to activate repairing processes for damage to DNA or proteins caused by UV-B (Buma et al. 2003; Gao et al. 2008). Therefore, the lower ratio of UV-B/PAR in winter (Figure 2A) could contribute to less UV-B inhibition of the carbon fixation than in summer (Table II). Lower UV-B inhibition of the carbon fixation in winter than in summer (Table II) was not observed in Wu et al. (2010).

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