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Comparison of the Effects of Short-Term UVB Radiation Exposure on Phytoplankton Photosynthesis in the Temperate Changjiang and Subtropical Zhujiang Estuaries of China

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We examined the effect of solar ultraviolet radiation B (UVB, 280–315 nm) on photosynthesis of natural phytoplankton assemblages in the temperate Changjiang River Estuary (CRE) in the East China Sea (ECS), and the subtropical Zhujiang River Estuary (ZRE) in the South China Sea (SCS) from August 2002 to April 2003. The short-term effect of UVB was assessed by exposing samples in quartz tubes/bottles to solar radiation under three treatments: (1) natural sunlight (NS) with UVB (NS-UVB); (2) photosynthetically active radiation (PAR, NS cut off UVB); and (3) NS with additional artificial UVB (NS + A-UVB). Solar UVB apparently inhibited phytoplankton photosynthesis rates. In the temperate CRE-ECS, solar UVB reduced surface phytoplankton photosynthesis by about 28% in August and February, while in the subtropical ZRE-SCS the inhibition was only 22% in September and October. In the CRE-ECS, phytoplankton in the stratified water column displayed stronger UVB inhibition when deeper water samples were exposed to surface UVB. Phytoplankton in the mixed water column did not show strong UVB inhibition, while light shift exposure of deeper phytoplankton in the same water column to surface light produced similar results, indicating that mixing moderates UVB effects. In the ZRE-SCS, surface phytoplankton showed greater photoinhibition in January (sunny). However, in April (cloudy), phytoplankton showed little UVB inhibition. Incubation for a short time without UVB showed a large increase in Chl *a* at two stations in the ZRE-SCS, but a large decrease at the other station in the presence of UVB. In contrast, in the CRE-ECS, a similar incubation experiment without UVB showed a decrease in Chl *a*, and small UVB inhibition of Chl *a* at two stations. Nutrient conditions might have played a role in the difference of UVB inhibition between the two regions as the ZRE-SCS had relatively high concentrations of all nutrients while PO_4 was only 0.21 μM at one of the CRE-ECS stations. The results suggest that phytoplankton in temperate waters would be more responsive to variation of UVB than ones in subtropical waters.

Keywords:
· UVB,
· phytoplankton,
· primary production,
· photoinhibition,
· Changjiang River Estuary-East China Sea,
· Zhujiang River Estuary-South China Sea.

1. Introduction

Increasing anthropogenic emissions of chlorofluorocarbons (CFCs) and nitrogen dioxide as a result of fertilizer application have induced stratospheric

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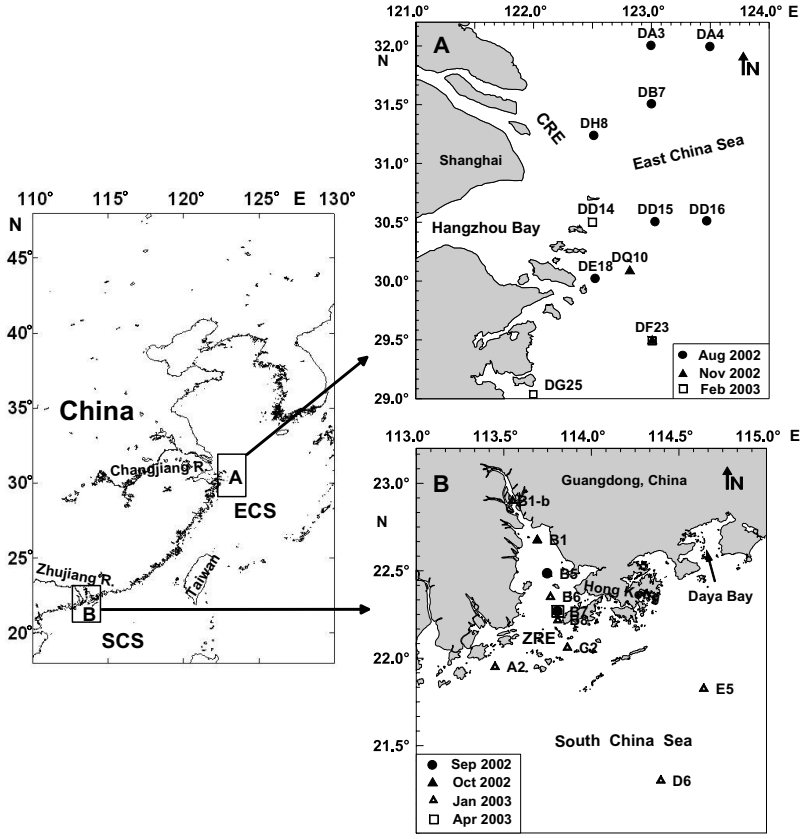

Fig. 1. Location of sampling stations in (A) Changjiang River Estuary-East China Sea (CRE-SCS) waters and (B) Zhujiang River Estuary-South China Sea (ZRE-SCS) waters.

ozone depletion (Bouwman, 1998). The emissions of greenhouse gases have also produced a decrease in stratospheric temperature, leading to enhanced formation of polar stratospheric clouds and this may increase ozone loss in polar regions (Shindell *et al*., 1998). Compared to 1979, measurable ozone losses have occurred over the Arctic and temperate regions (Villafañe *et al*., 2004; Hogue *et al*., 2005). Some depletion has been observed over tropical regions, although it is not statistically significant (WMO, 1999; Rozema *et al*., 2002).

Ozone depletion has increased the flux of biologically damaging solar ultraviolet radiation B (UVB, 280–315 nm) to the surface of the earth (Neale *et al*., 1998). UVB penetrates to depths >30 m in clear oceanic waters, while in productive regions it attenuates more rapidly (Smith and Baker, 1979; Behrenfeld *et al*., 1993). Increased solar UVB may result in changed species composition and ecosystem integrity (Häder, 2000), affecting the food web community structure (Häder *et al*., 1995), decreasing the biomass productivity of aquatic ecosystems (Villafañe *et al*., 2004), reducing the sink capacity for atmospheric carbon dioxide (Takahashi *et al*., 1997), and possibly reducing food production for humans (Häder, 2000).

The oceans produce a plant biomass, similar to terrestrial ecosystems (Smith, 1989). Solar UVB inhibits the photosynthetic rates of phytoplanktonic organisms (Smith and Cullen, 1995) by affecting both light-limited and light-saturated carbon uptake (Lesser, 1996). Most studies focus on polar and temperate regions, and UVB inhibits carbon incorporation in field populations of temperate and Antarctic phytoplankton by 25 to 50% (Maske, 1984; Neale *et al*., 1998; Villafañe *et al*., 2004). Studies on tropical marine environments are rare (Behrenfeld *et al*., 1993; Helbling *et al*., 2003; Gao *et al*., 2007a, b).

The Changjiang (Yangtze) River and the Zhujiang (Pearl) River are the two largest rivers in China, ranking 3rd and 13th, respectively, in the world according to discharge volume. The Changjiang River drains in the temperate zone, while the Zhujiang River is in the subtropical region. The Changjiang River provides 85% of the freshwater runoff to the East China Sea (ECS). Twenty nine percent of the runoff occurs in the dry season (November to April), and 71% during the wet season (May to

Table 1. Regions, dates, sampling stations and experiments in the Changjiang River Estuary-East China Sea (CRE-ECS) and Zhujiang River Estuary-South China Sea (ZRE-SCS).

Region	Date	Station	Experiment
CRE-ECS	Aug. 22–Sep. 11, 2002	DA3, DA4, DB7, DH8, DD15, DD16, DE18	A
	Nov. 5–10, 2002	DQ10, DF23	B
	Feb. 25–Mar. 10, 2003	DD14, DF23, DG25	C
ZRE-SCS	Sep. 27, 2002	B5, B7	A, B
	Oct. 22, 2002	B1	A
	Jan. 14–15, 2003	A2, B1-b, B6, B8, C2, D6, E5	D, E, F
	Apr. 12, 2003	B7	D

Experiments are denoted as: A, UVB effects on primary productivity; B, time course incubation; C, light shift experiments; D, photosynthesis-irradiance (P-E) curves; E, water column irradiance profiles; F, acclimation experiments.

October) (Zhang, 1996). The discharge is rich in nitrate and silicate but low in phosphate. The annual flux of nitrate and silicate from the Changjiang River to the ocean are about 6×10^{10} and 12×10^{10} mol, respectively (Edmond *et al*., 1985; Zhang, 1996). The Zhujiang River carries 3.5×10^{11} m^3yr^{-1} of fresh water with a sediment load of 85×10^6 tons yr^{-1} into the South China Sea (SCS), 70–80% of the discharge occurs during the wet season (April to September) and 20–30% during the dry season (October to March) (Yin *et al*., 2001). There is evidence that indicates that P limitation occurs both in the Changjiang River and Zhujiang River estuaries, while N is limiting offshore (Harrison *et al*., 1990; Yin *et al*., 2001).

There is no information about the effects of UVB on the photosynthesis of phytoplankton assemblages during their vertical migration (mixing) in the water column of the main typical Chinese estuarine waters, such as Changjiang River Estuary (CRE) or Zhujiang River Estuary (ZRE) waters. No comparison of UVB effects between two large estuaries has hitherto been done, and this study also makes a significant contribution to understanding the ecological comparison between temperate vs. subtropical waters. The objectives of the present study are: (1) to describe the spatial variation of UVB in the water column from the turbid river mouth to adjacent coastal waters in the ZRE-SCS; (2) to assess UVB inhibition of photosynthesis in phytoplankton organisms of different cell sizes and in both temperate and subtropical estuarine waters; and (3) to explore if there is a relationship between low nutrient conditions and UVB inhibition.

2. Materials and Methods

2.1 Study area

The East China Sea (ECS) is influenced by the Changjiang River estuarine (CRE) waters and the Zhujiang River estuary (ZRE) opens to the northern part of the South China Sea (SCS) (Fig. 1). Several cruises have been conducted in the CRE-ECS (Fig. 1A) in August and November 2002 and February 2003 (Table 1), and in the ZRE-SCS (Fig. 1B) in September and October 2002 and January and April 2003 (Table 1).

2.2 Field water sampling and investigation

A YSI 6600 instrument (YSI Inc., Yellow Spring, OH, USA) was used at a station to take vertical profiles of temperature and salinity. Water samples were collected using Niskin bottles at the surface or middle layer, and subsamples were transferred into acid-cleaned polycarbonate bottles for collecting Chl *a*, nutrient samples and conducting field incubation experiments.

2.3 Short-term field incubation experiments

Water samples for primary productivity (PP, ^{14}C uptake) incubations were prescreened through a 200 μm mesh net to remove larger zooplankton, and then transferred to 50 ml quartz tubes, inoculated with 2 μCi labeled sodium bicarbonate ($NaH^{14}CO_3$) and incubated for 4–6 h between 08:00 and 16:00 h. All experimental treatments were in duplicate and the tubes were placed horizontally (i.e., parallel to the water surface) in a water bath on board the ship. The horizontal placement helped to mix the incubated water samples. The bath was flushed with surface sea water to maintain in situ temperature.

2.3.1 Treatments

Duplicate 50 ml quartz tube (or 2 L quartz bottle) samples were incubated under: (a) NS-UVB (natural sunlight with UVB); (b) PAR (natural sunlight without UVB), achieved by shielding UVB with Mylar film (Melinex film 389, Dupont products); and (c) NS + A-UVB (natural sunlight plus additional artificial UVB). The artificial UVB source was a SANKYO DENKI G6T5E UVB lamp (SANKYO Co., Tokyo, Japan), which is a fluorescent bulb

emitting ultraviolet rays in the 280–360 nm wave band with a peak at 306 nm and ultraviolet output 1.0 W.

2.3.2 Short-term time course incubation

Two L quartz bottles were used for 4 h time course incubation of surface water under treatments (a) and (b) at Stns DF23 and DQ10 in CRE-ECS and Stns B5 and B7 in PRE-SCS (Table 1). Samples of initial Chl *a*, and nutrients (NO_3, PO_4 and SiO_4) were collected. During the 4 h incubation, Chl *a* samples were collected hourly to detect any short-term impact of natural UVB on phytoplankton biomass.

2.3.3 Light shift experiments

In the CRE-ECS waters we took water samples at the surface and the middle of the water column at Stns DG25, DF23 and DD14 (6, 30 and 14 m deep, respectively). The samples under treatments (a), (b) and (c) were incubated in quartz tubes under simulated light conditions at the depths from which samples were collected. The artificial UVB radiation doses were ~2.5 times (~0.5 W m^{-2}) ambient solar UVB intensity at these three stations. Shifted light conditions mean that the surface water samples were incubated at middle depth light intensities, while the middle water samples were irradiated under at surface light only under treatments (a) and (b). The simulated middle depth light and additional artificial UVB conditions were achieved with different layers of neutral density screens.

2.3.4 Photosynthesis-Irradiance (P-E) curves

P-E curves for surface water samples of Stns E5, A2 and B7 in ZRE-SCS waters were obtained by conducting ^{14}C uptake experiments in quartz tubes (Table 1). The incubations were carried out under different weather conditions (i.e., Stns E5 and A2, sunny and Stn B7, cloudy) after three treatments (a), (b) and (c) mentioned above. The artificial UVB doses were controlled at ~2 times the ambient solar UVB. A gradient of irradiance levels was achieved with neutral density screens, with a light penetration of 0% (dark, covered with aluminum foil), 10%, 30%, 50%, 70%, 85%, and 100% (uncovered).

2.3.5 Acclimation of different size phytoplankton to UVB

Surface water samples from Stn E5 (offshore) and Stn A2 (estuarine) were filtered through 5 μm polycarbonate membranes (Poretics, Osmonics Inc., MN, USA) in ZRE-SCS waters (Table 1 and Fig. 1B). Both the filtrate (phytoplankton size < 5 μm) and the whole water samples were incubated under treatment (a) and (b) using ^{14}C tracer labeling technique. A 300 ml water sample was filtered onto a 25 mm Whatman GF/F glass fiber filter (pore size 0.7 μm, diameter 25 mm, Whatman Inc., Clifton, NJ, USA) for initial total Chl *a*, or a 500 ml sample for phytoplankton size fractionation was filtered through 5 μm polycarbonate membranes and the filtrate was then filtered through a Whatman GF/F filter to collected phytoplankton <5 μm.

2.4 Measurements and analyses of samples

2.4.1 Radiation measurement

Photosynthetically active radiation (PAR, 400–700 nm) and UVB dose in the air were measured with submersible, broad-band, and photodiode PAR and UVB detectors attached to an IL-1700 Research Radiometer (International Light Inc., MA, USA). PAR and UVB profiles in the water column were measured in January 2003 in the ZRE-SCS. These profile measurements were not done in the CRE-ECS waters due to instrument breakdown. Vertical penetration of UVB and PAR in the water column is subject to the exponential attenuation law, $I_Z = I_0 e^{-KZ}$, where I_Z and I_0 are the light intensity at a depth Z and at the surface, respectively. K (m^{-1}) is the light extinction coefficient. K_{PAR} and K_{UVB} are the extinction coefficients for PAR and UVB, respectively. The 1% light level depth of PAR and UVB were calculated from K_{PAR} and K_{UVB} (Yin *et al*., 2004).

2.4.2 Photosynthetic rates

After the incubation, samples from the ^{14}C addition experiments were filtered through Whatman GF/F filters mounted on a PALL filter funnel (Gelman Laboratory Inc., MI, USA), and the filters were kept frozen (–20°C) until they were analyzed within one month following the JGOFS protocols (Knap *et al*., 1996). The filters were put into scintillation vials containing 0.2 ml of 0.5 N HCl for 12 h to remove inorganic carbon. After the addition of 10 ml of scintillation cocktail (Hi-Safe) to each vial, samples were counted on a Beckman 2000 CA/LL Liquid Scintillation Counter and primary productivity (^{14}C uptake rates) were calculated according to standard procedures (Knap *et al*., 1996). The relative photoinhibition ratio due to UVB was calculated as follows:

$$\text{Inh}\ (\%) = (P_{\text{no UVB}} - P_{\text{UVB}})/P_{\text{no UVB}} \times 100\% \qquad (1)$$

where $P_{\text{no UVB}}$ represents the amount of carbon fixed in the no UVB treatment and P_{UVB} is the carbon fixed in the NS-UVB or intensified artificial UVB treatment (Yuan *et al*., 2007). The chlorophyll-specific photosynthetic rates P^B (mg C (mg Chl *a*)$^{-1}h^{-1}$), also referred to as the assimilation number, AN, were calculated from primary productivity divided by phytoplankton biomass (Chl *a*).

2.4.3 Analysis of chlorophyll a and nutrients

Chl *a* was measured fluorometrically after the method of Parsons *et al*. (1984). Chl *a* samples were stored in aluminum foil and frozen immediately at –20°C for laboratory analysis onshore, which was carried out within 15 d. Chl *a* was extracted with 10 ml 90% acetone, the filters in acetone were sonicated for 10 min in an ice-cold water bath in the dark, and were placed at 4°C in the dark for 24 h. The fluorescence of the extract was measured with a Turner Designs TD-700 fluorometer. The filtrate (30 ml) of precombusted (450°C, 5 h) Whatman GF/F

filter was collected and frozen for nutrient analysis in the laboratory. Nutrients (NO_3, PO_4 and SiO_4) were analyzed with a Skalar San Autoanalyzer according to the colorimetric methods described in Yin *et al*. (2001).

2.4.4 Data analysis

A one-way analysis of variance (ANOVA) was used to determine a significant difference among the treatments at the probability $P < 0.05$ level.

3. Results and Discussion

3.1 PAR and UVB profiles

The ambient incident light intensity at Stns B1-b, B6, B8, C2, E5 and D6 was 157, 39, 194, 236, 64, and 115 W m^{-2} for PAR, and 0.11, 0.01, 0.09, 0.20, 0.02, and 0.06 W m^{-2} for UVB, respectively. Both PAR and UVB decreased rapidly in the ZRE-SCS waters (Fig. 2). The attenuation coefficient of PAR, K_{PAR}, decreased from 1.85 m^{-1} at the river mouth (Stn B1-b) to 0.23 m^{-1} at Stn D6 in the northern SCS, while K_{UVB} was 9.45 m^{-1} at Stn B1-b and 0.39 m^{-1} at Stn D6 (Fig. 2A). As shown in Fig. 2B, the 1% penetration level was much lower for UVB (0.5 m) than PAR (2.5 m) at Stn B1-b in the turbid estuarine waters, while in the oceanic environment the 1% light depth penetrated to ~16 m and 20 m for UVB and PAR at Stn D6, respectively.

The ratio of the 1% depth of UVB to PAR can be used to estimate the potentially detrimental effects on algae of solar UVB radiation in the euphotic zone (Piazena and Häder, 1994). The larger the ratio is, the more the algae may be damaged. The ratio was 0.2:1 at the river mouth, increasing to 0.75:1 in the oceanic waters (Fig. 2C). These results are similar to the observations of Smith and Baker (1979), who found that the attenuation coefficient, K_{UVB}, of UVB penetration in natural waters varied over a wide range, from 20 m^{-1} in turbid waters in estuaries to <0.2 m^{-1} in the clear ocean waters. Biologically effective levels of solar UVB could penetrate to at least 30 m depth in oceanic waters (Holm-Hansen *et al*., 1993), whereas in a coastal lagoon of the Southern Baltic Sea the levels were <1 m (Piazena and Häder, 1994). Rapid UVB attenuation is mainly caused by dissolved and particulate absorbing substances, including colored dissolved organic matter (CDOM, or yellow substance), Chl *a* and other photosynthetic pigments, as well as organic and inorganic particulate matter (Chen *et al*., 2002). In the Pearl River estuary, the river water is the main source of CDOM, the highest levels of CDOM have been found in fresh water, and the lowest in sea water (Chen *et al*., 2004).

3.2 Inhibition of carbon fixation by UVB

Carbon uptake in the CRE-ECS waters ranged between 1 and 20 mg C $m^{-3}h^{-1}$ under NS without UVB. In

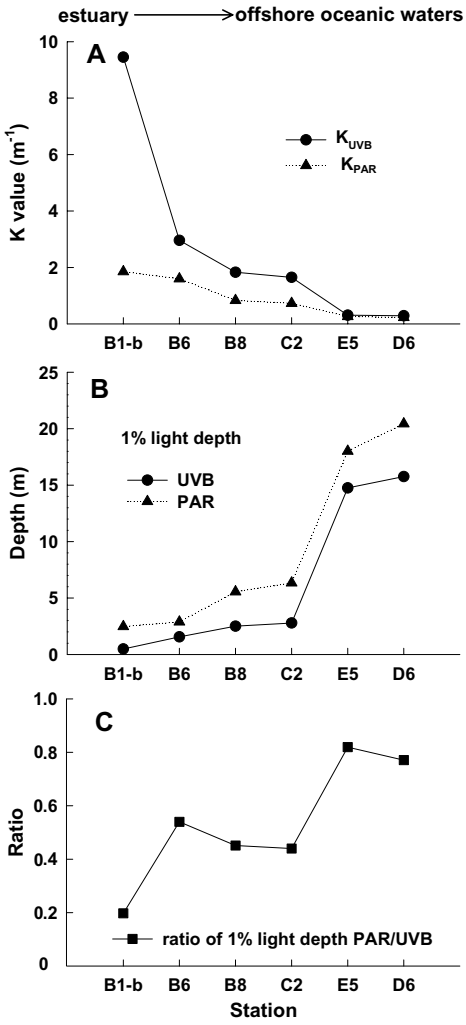

Fig. 2. (A) Extinction coefficient, K_{PAR}, for PAR and K_{UVB}, for UVB; (B) 1% light depth of PAR and UVB; and (C) the ratios of 1% light depth of UVB to PAR, along the ZRE to SCS in January 2003.

August, average C uptake for surface water phytoplankton under solar UVB was reduced by 28.4%, ranging from 10 to 80%, compared to C uptake with no UVB. In the ZRE-SCS waters, C uptake was of a similar magnitude under NS without UVB and was reduced on average by 22.2% by UVB during September to October (Fig. 3A). Helbling *et al*. (2003) found 24% mean photoinhibition off the southeast China coast during May 2002. C uptake for Stn DE18 was low because it was influenced by turbid CRE water, comparable to Stn B1 in the ZRE (Fig. 3). In general, organisms from high latitudes show higher net damage due to NS UVB. Vernet (2000) compared photoinhibition by natural solar UVB in surface phytoplankton in three different coastal waters during spring and found that Antarctic phytoplankton showed

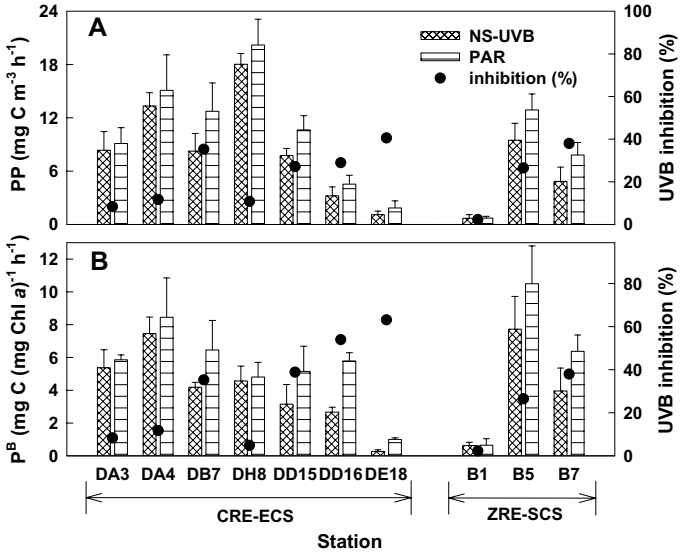

Fig. 3. (A) Primary productivity (PP), (B) chlorophyll-specific photosynthetic rates P^B and UVB inhibition at stations in the CRE-SCS and the ZRE-ECS. The closed circle between two bars represents UVB inhibition (%) on PP and P^B at that station. NS-UVB in the figure legend represents natural sunlight as the incubation light, and PAR as NS without UVB.

greater photoinhibition than temperate and tropical phytoplankton. Spring phytoplankton populations at the surface in the Antarctic showed an increase of 50% in carbon incorporation after screening of UVB (Holm-Hansen *et al*., 1993). Temperate surface phytoplankton in Valparaiso Bay, central Chile, increased carbon incorporation by only 4% upon screening of UVB (Helbling *et al*., 2001). Tropical phytoplankton from the mixed layer did not show an increase in C uptake after screening of UVB (Helbling *et al*., 1992). The difference suggests a better adaptation of phytoplankton at lower latitudes to UVB, either because of a greater tolerance to higher subtropical UVR or an increased rate of repair of UV damage.

The chlorophyll-specific photosynthetic rates P^B, in the CRE-ECS were 3.95 ± 2.26 (with a range of 0.27–7.46) and 5.36 ± 2.26 (1.00–8.45) mg C (mg Chl *a*)$^{-1}$h^{-1} with and without UVB, respectively, in August 2002. In the ZRE-SCS, the average value of P^B were 4.10 ± 3.55 (0.63–7.72) and 5.84 ± 4.95 (0.64–10.50) mg C (mg Chl *a*)$^{-1}$h^{-1}, with and without UVB, respectively, during September to October (Fig. 3B). On average, C fixation based on Chl *a* decreased by 33.0% in the CRE-ECS and 29.8% in the ZRE-SCS due to the UVB inhibition. However, the inhibition expressed by P^B under NS-UVB in the ZRE-SCS was similar to that expressed in C uptake (mg C m^{-3}h^{-1}), whereas the inhibition in the CRE-ECS was greater. Since P^B is an indication of phytoplankton Chl *a* photosynthetic efficiency, higher UVB inhibition in CRE-ECS than ZRE-SCS suggests that subtropical phytoplankton are better adapted to UVB.

Table 2. Initial Chl *a*, nutrients and N:P ratios (DIN:PO_4) for the time course of incubation at Stns DF23 and DQ10 (November 2002) in the CRE-ECS, and at Stns B5 and B7 (September 2002) in the ZRE-SCS.

Station	Chl *a* (mg m^{-3})	NO_3 (μM)	PO_4 (μM)	SiO_4 (μM)	N:P ratio
DF23	0.87	1.69	0.21	4.98	20.5
DQ10	1.38	6.09	0.40	13.03	17.4
B5	1.23	76.19	0.94	82.66	91.2
B7	1.23	32.43	0.56	36.76	61.5

3.3 Short-term incubation: nutrients effects

UV damage to chlorophyll or chloroplasts is the principal cause of the inhibition effect (Smith and Cullen, 1995). Chl *a* concentration decreased during 4 h incubation with and without UVB for samples at Stns DF23 and DQ10 in the CRE-ECS, but the decrease was greater with UVB (Fig. 4). The linear regression equations of Chl *a* against incubation time (*T*) for treatments with UVB and without UVB at these two stations were:

For Stn DF23:

$$\text{Chl } a = -0.187 \times T + 0.716 \ (P < 0.01), \text{ NS-UVB;} \quad (2)$$

$$\text{Chl } a = -0.185 \times T + 0.750 \ (P < 0.01), \text{ PAR (without UVB).} \quad (3)$$

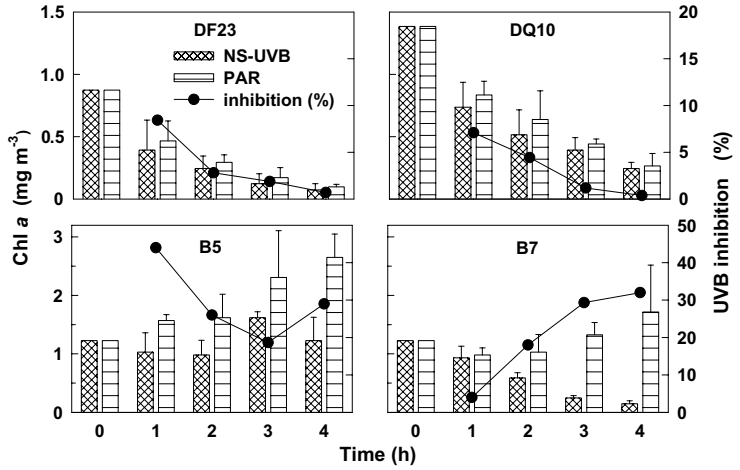

Fig. 4. Changes in Chl *a* and UVB inhibition during the 4 h incubation of surface water samples collected at Stns DF23 and DQ 10 in the CRE-ECS, and Stns B5 and B7 in the ZRE-SCS. The filled circles between two bars represent UVB inhibition (%) on Chl *a* changes with incubation time at that station. NS-UVB in the figure legend represents natural sunlight as the incubation light, and PAR as NS without UVB.

Table 3. Temperature, salinity, initial Chl *a*, initial nutrients and UVB inhibition of different treatments for the light shift experiment at Stns DG25, DF23 and DD14 (February 2003) in the CRE-ECS.

Station	DG25 (12 m)		DF23 (60 m)		DD14 (28 m)	
Layer	S	M	S	M	S	M
Sampling depth (m)	0	6	0	30	0	14
Temperature (℃)	9.8	9.7	12.4	12.4	9.4	9.3
Salinity	27.1	27.1	32.9	33.0	25.5	26.9
Chl *a* (mg m^{-3})	0.27	0.28	0.28	0.27	0.42	0.47
NO_3 (μM)	15.82	9.23	3.10	4.99	24.21	15.70
PO_4 (μM)	0.48	0.42	0.31	0.42	0.35	0.24
SiO_4 (μM)	22.61	10.30	6.54	9.37	27.30	17.83
N:P ratio	35.8	27.5	15.9	17.2	74.7	79.0
UVB inhibition (%)						
A: original light	30.0	14.4	7.7	6.3	44.3	37.1
B: original light + A-UVB	57.2	42.9	44.6	15.7	62.8	52.9
C: shift light	34.2	18.2	10.0	3.9	29.7	40.8

S and M denote water sample collected at surface (S) and middle (M) layer, respectively. A: incubation light condition of S and M samples were under simulated sunlight conditions at the original depths where the samples were collected; B: the simulated light condition at original depths with additional artificial UVB (A-UVB); C: shift light condition for S and M layer, i.e., the S sample was placed under the simulated M light condition and the M sample under the surface light condition.

For Stn DQ10:

$$\text{Chl } a = -0.262 \times T + 1.179 \ (P < 0.01), \text{ NS-UVB}; \quad (4)$$

$$\text{Chl } a = -0.263 \times T + 1.239 \ (P < 0.01), \text{ PAR (without UVB)}. \quad (5)$$

The slopes between treatments with and without UVB, i.e., Eqs. (2) and (3), Eqs. (4) and (5), were not significantly different. The ratio of the decrease in Chl *a* with UVB to that without UVB (% inhibition) was <10% initially and decreased further during the 4 h incubation, suggesting that UVB inhibition disappeared at Stns DF23 and DQ10. In contrast, Chl *a* increased at Stns B5 and B7 without UVB, but remained relatively constant (Stn B5) or decreased steadily (Stn B7) with UVB (Fig. 4). The inhibition of UVB increased at Stn B7. The differences between the CRE-ECS and ZRE-SCS stations might be

related to nutrient conditions. Compared to the ZRE waters (Stns DF23 and DQ10), initial nutrients were far lower at the CRE-ECS stations (Stns B5 and B7) (Table 2). PO_4 was only 0.21 μM at DF23. The inhibition by UVB was greater in the ZRE-SCS than in the CRE-ECS. Thus, the lower UVB inhibition at Stns DF23 and DQ10 appears to suggest that the stress from low nutrients might have alleviated UVB inhibition (note: lower turbidity should allow greater UV penetration). In addition, the gentler slope at Stn DQ10 than at Stn DF23 indicates a slower UVB effect at Stn DQ10 as nutrients at the former station were higher than at the latter (Fig. 4). However, a series of week-long mesocosm experiments in Rimoushi (Canada) indicated that nitrite addition partly relieved UVB inhibition only during the post-bloom period (Longhi *et al.*, 2006). Factors such as temperature, mixing, grazing and the dominant species in different regions might have played some roles.

3.4 Light shift: stratification versus mixing

When wind- or tide-induced vertical mixing (upwelling/downwelling) occurs in a stratified water column, phytoplankton at different depths in the water column will be exposed to stronger or lower light. Phytoplankton may use different strategies to cope with UVB radiation: different light-adaptation abilities or better recovery from the damage inflicted by UVB radiation (Kishino *et al.*, 1985).

Chl *a* at Stns DG25, DF23 and DD14 in the CRE-ECS was low, <0.5 mg m^{-3}, ranging from 0.27 to 0.47 mg m^{-3} in February 2003, with a higher Chl *a* concentration at Stn DD14 near the Hangzhou Bay (Fig. 1 and Table 3). The average incident sunlight PAR was 161, 169 and 174 W m^{-2} for Stns DG25, DF23 and DD14, respectively, during the incubation. While the average incident sunlight UVB and intensified UVB were 0.21 and 0.51 W m^{-2}, 0.18 and 0.46 W m^{-2}, 0.24 and 0.56 W m^{-2} for Stns DG25, DF23 and DD14, respectively. Sunlight conditions during the incubation of water samples for these three stations were almost similar. The intensified UVB was close to 2.5 times the ambient sunlight UVB.

UVB inhibition of phytoplankton primary productivity was on average 23.3% (ranged from 6.3 to 44.3%) at Stns DG25, DF23 and DD14 in the CRE-ECS (Table 3). When exposed to the added UVB, the inhibition was almost doubled at all three stations, and exceeded 60% at the surface of Stn DD14. Phytoplankton at the middle depth at Stn DF23 showed the lowest UVB inhibition compared to the other two stations under any treatments (Table 3 and Figs. 5D–F). Stn DF23 was dominated by strongly mixed oceanic water (salinity ~33 at the surface and middle of the water column) (Fig. 5B). As a result, photosynthetic rates were similar between surface and middle depths and UVB inhibition changed little when

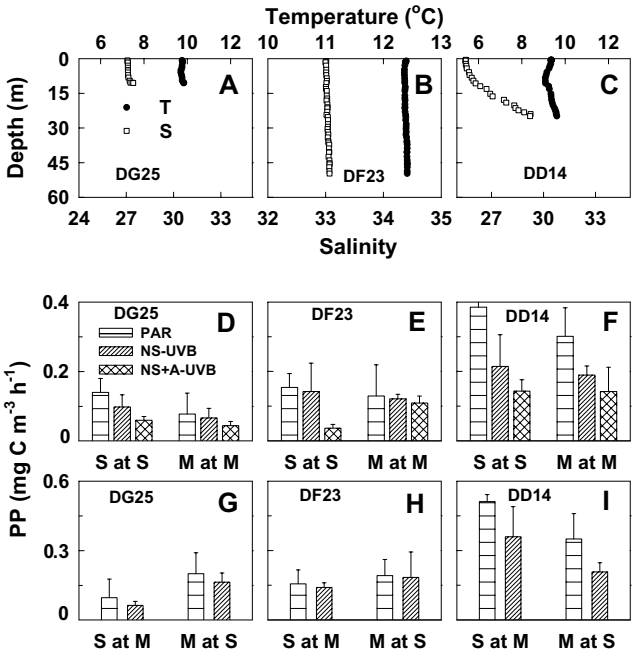

Fig. 5. (A–C) Profiles of temperature and salinity; (D–F) photosynthetic rates in the water column at Stns DG25, DF23 and DD14 in February 2003 in the CRE-ECS, in which the water samples were incubated under simulated in situ light intensity conditions: S at S means the surface layer (S) samples incubated under the surface light condition, M at M means the middle layer (M) samples under middle layer light condition; and (G–I) photosynthetic rates for the S samples incubated under the M light condition (S at M) and the M samples under the S light condition (M at S). NS-UVB in the figure legend represents natural sunlight as the incubation light, PAR as NS without UVB, and NS + A-UV-B as natural sunlight with artificial UVB.

phytoplankton in the water samples from the middle depth were exposed to the surface ambient light intensity dose (Figs. 5E and H). Photosynthesis at Stns DG25 and DD14 were more strongly inhibited when phytoplankton from the deeper layer were exposed to the surface sunlight, particularly at Stn DD14, where the water column was deeply stratified (Figs. 5G and I). Vertical mixing may have increased UVB tolerance of phytoplankton at Stn DF23 as they had been exposed to high UVB in the course of vertical mixing (Gustavson *et al.*, 2000).

3.5 P-E curves: sunny versus cloudy

Profiles of temperature and salinity at Stns E5, A2 and B7 showed that the surface water at Stn E5 was dominated by oceanic waters, while Stns A2 and B7 were dominated by Zhujiang River estuarine waters (Figs. 6A–C). Chl *a* concentration was high at Stn A2, almost five times higher than at Stn B7 and 12 times higher than at Stn E5

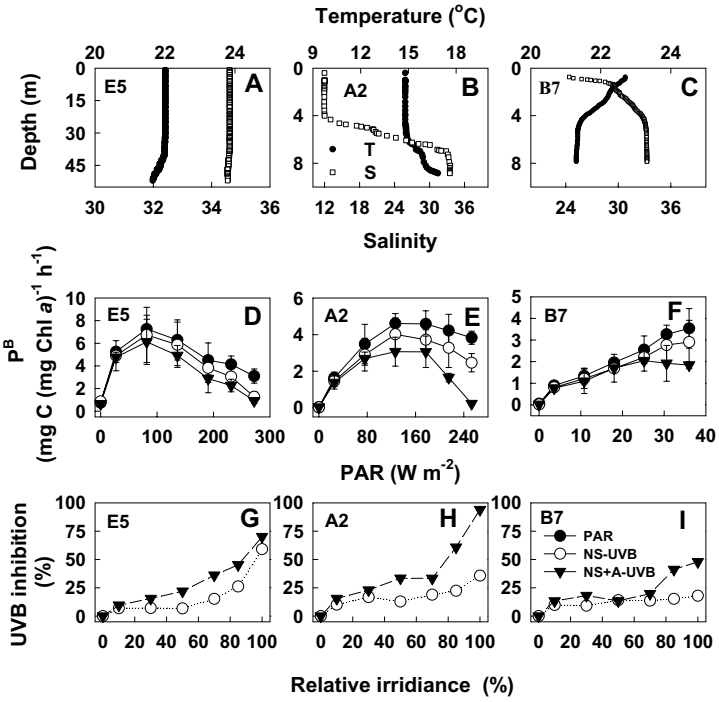

Fig. 6. (A–C) Profiles of temperature and salinity; (D–F) Chlorophyll-specific photosynthetic rates P^B vs. PAR irradiance (P-E) curves; and (G–I) UVB inhibition (%) of P^B vs. relative irradiance (%) for surface water samples at Stns E5 and A2 in January and Stn B7 in April 2003 in the ZRE-SCS. The upper three plots show the profiles of temperature and salinity. NS-UVB in the figure legend represents natural sunlight as the incubation light, PAR as NS without UVB, and NS + A-UV-B as natural sunlight with artificial UVB.

(Table 4). Nutrients in the surface water were low at Stn E5, with PO_4 being only 0.22 μM, about one quarter of the value at Stn B7 (Table 4).

In the course of P-E curve incubation, the average ambient PAR was 272, 253 and 36 W m^{-2} for Stns E5, A2 (sunny) and Stn B7 (cloudy) in the ZRE-SCS, respectively, while the average added UVB intensities were 0.72 and 0.73 W m^{-2} for Stns E5 and A2, nearly twice as high as ambient solar UVB. The P-E curves for Stns E5 and A2 showed strong effects of UVB on photosynthesis under the three treatments when PAR was >30% of incident solar radiation due to the presence of high UVB levels on the sunny day (Figs. 6D, E, G and H). At Stn B7, the ambient solar light was not strong enough to achieve the light-saturated condition for phytoplankton photosynthesis, as the P-E curve had not shown the saturation plateau at 100% natural light intensity under cloudy weather, except for the added UVB treatment, which reached 0.45 W m^{-2} (Fig. 6F). Only the relative light intensity more than 85% of the added UVB treatment showed apparent inhibition. Smith and Cullen (1995) reported a UVB threshold of 0.5 W m^{-2} for photosynthetic inhibition to phytoplankton in Antarctic coastal waters. The ambient solar UVB at Stn B7 was much lower, with the average value of 0.02 W m^{-2} during the incubation due to cloudy weather, and hence the natural ambient solar UVB showed little inhibition of phytoplankton under all the irradiance ratio treatments (Fig. 6I). UVB inhibition of C uptake by natural sunlight at Stn E5 was close to Stn A2 (Table 4), but the average percentage of UVB inhibition at Stn E5 was lower than Stn A2 under intensified UVB treatment. This might be caused by: (1) phytoplankton at Stn E5 were under low nutrients and UVB inhibition might be alleviated under nutrient stress, and more research is required to test this hypothesis; (2) the smaller phytoplankton had stronger adaption mechanisms to an increase in UVB, as demonstrated in size fractionation experiments reported in the next section.

3.6 Phytoplankton composition and photo-acclimation of ultraphytoplankton (<5 μm)

The microplankton species were mainly represented by the diatom genera *Coscinodiscus*, *Pleurosigma* and *Pseudonitzschia* in the CRE-ECS waters, while in the ZRE-SCS waters the main diatom genera were *Chaetoceros*, *Rhizosolenia*, *Pseudonitzschia*, *Coscinodiscus* and *Skeletonema* during the study period.

Pico- and nanophytoplankton, size <~5 μm, defined

Table 4. Temperature, salinity, initial Chl *a*, initial nutrients, and average UVB inhibition (%) of surface samples under 0%, 10%, 30%, 50%, 70%, 85% and 100% ambient irradiance levels of different UVB treatments for P-E curves at Stns E5, A2 (January 2003) and Stn B7 (April 2003) in the ZRE-SCS.

Station	E5 (61 m)	A2 (10 m)	B7 (8 m)
Temperature (℃)	22.0	14.8	22.7
Salinity	34.6	12.0	24.4
Chl *a* (mg m^{-3})	0.45	5.58	1.09
NO_3 (μM)	1.63	55.23	57.29
PO_4 (μM)	0.22	0.55	0.97
SiO_4 (μM)	3.19	83.44	54.80
N:P ratio	43.3	146.0	83.4
Average UVB inhibition (%)			
A: NS-UVB	20.2	19.4	13.3
B: NS + A-UVB	33.1	43.3	25.6

A: incubation light condition of different light penetration series tubes were under simulated natural sunlight conditions (NS-UVB); B: the simulated natural sunlight condition with additional artificial UVB (NS + A-UVB).

as "ultraphytoplankton" (Murphy and Haugen, 1985), are numerically dominant in open oceanic waters. At the offshore stations (Stns DA4, DD16, DF23 in CRE-ECS and Stns E5, D6 in ZRE-SCS), Chl *a* was dominated by the >5 μm size phytoplankton (>50%), but at other near shore stations Chl *a* was dominated by the <5 μm ultraphytoplankton.

During January 2003 in the ZRE-SCS, ultraphytoplankton exceeded 70% of the total biomass at Stn E5 (offshore), while at the river mouth, phytoplankton >5 μm dominated at Stn A2 (Table 5). Size-fractionated phytoplankton incubated under NS with and without UVB showed that ultraphytoplankton had a better adaptation to UVB. Only ~13% inhibition ratio to ultraphytoplankton primary productivity was detected at offshore Stn E5, while at the estuarine Stn A2 the inhibition reached 30% (Table 5). The difference in UVB inhibition demonstrates that the phytoplankton experienced more UVB exposure in oceanic waters and hence less UVB inhibition, whereas the estuarine phytoplankton received turbidity-reduced UVB and hence were more sensitive to UVB exposure. Small natural phytoplankton assemblages such as picophytoplankton (<2 μm) are better adapted to the harsh environmental conditions (Li and Luan, 1998). In a subarctic pelagic ecosystem, cells >2 μm were twice as sensitive to solar UVB as smaller cells (Milot-Roy and Vincent, 1994). Helbling *et al*. (2001) indicated that, although small cells are more susceptible to DNA damage, they are more resistant to photosynthesis inhibition. The relation was not always present, though, in contrast, different results were obtained by Karentz *et al*. (1991) in a incubation experiment on 12 species of Antarctic diatoms. They suggested that smaller cells sustained more damage per unit of DNA and were more sensitive to UV exposure, since small cells have higher surface area to volume ratios than large ones, and they receive more exposure to UV than larger cells under the same light. Wängberg *et al*. (1996) found no clear relation between cell size and sensitivity to UVB radiation. In addition to the size, the different eco-physiological characteristics between indoor culture and natural assemblages of phytoplankton and the different content of UV screen pigment among algal species were also important factors affecting photo-acclimation to UVB.

Table 5. Photoinhibition ratios of total phytoplankton and ultraphytoplankton (<5 μm) primary productivity by natural sunlight UVB at an estuarine station E5 and an oceanic station A2 in the ZRE-SCS in January 2003.

Station	$T_{Chl\,a}$ (mg m^{-3})	P_{ultra} (%)	Inhibition by NS-UVB (%)	
			$T_{Chl\,a}$	Ultra-
E5	0.45	72.0	18.0	12.9
A2	5.58	22.8	40.7	30.4

$T_{Chl\,a}$ denotes the total phytoplankton biomass (Chl *a*), and P_{ultra} denotes the proportion of ultraphytoplankton (<5 μm) biomass to $T_{Chl\,a}$. Ultra- is ultraphytoplankton.

4. Conclusions

In the temperate CRE-ECS waters, solar UVB inhibited surface phytoplankton photosynthesis by ~28% in summer and winter. In the subtropical ZRE-SCS waters, the natural sunlight UVB dose is much stronger than that in temperate regions, but the mean inhibition of sur-

face phytoplankton was only 22% from September to October. Phytoplankton in the CRE-ECS also experienced greater photoinhibition when exposed to artificial UVB. When the water column was mixed, phytoplankton displayed little UVB inhibition, indicating the adaptation of phytoplankton during vertical mixing (upwelling/downwelling). Our study provides important information on how the temperate CRE-ECS ecosystem would respond more strongly to variation in solar UVB radiation than the ZRE-SCS ecosystem, which has implications for the modeling of primary productivity in China seas in response to UVB variation due to climate change.

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