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Phytoplankton are more tolerant to UV than bacteria and viruses in the northern South China Sea

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ABSTRACT: In late summer of 2005, the effects of ultraviolet radiation (UVR) on primary production (PP), bacterial production (BP) and viral decay rates (VDR) were investigated along a salinity gradient in the northern South China Sea. The freshwater input increased the UVA diffuse attenuation coefficient (up to 2.7 m^{-1}) near the Pearl River estuary and consequently influenced the UVR inhibitory effects, as VDR was significantly correlated with the UVA diffuse attenuation coefficient. UVR inhibition of PP was significantly higher in upwelled waters than downwelled waters, suggesting that the vertical mixing (i.e. upwelling and downwelling) was an important factor regulating microbial sensitivity to UVR. UVR inhibited PP and BP by ~15 and 25%, respectively, and the UVR inhibition of BP was significantly higher than that of PP in most of the samples; hence there was a competitive advantage for phytoplankton over bacteria under the relatively high UVR exposure. UVR increased VDR by ~30%, which decreased bacterial mortality by 0.12 to 0.3% h^{-1} and mitigated the inhibitory effects of UVR on bacteria. In general, phytoplankton were more tolerant than bacteria and viruses to UVR in the sub-tropical northern South China Sea.

KEY WORDS: Bacterial production · Primary production · Viral decay · Ultraviolet radiation · South China Sea

INTRODUCTION

Solar ultraviolet radiation (UVR, 280 to 400 nm) is an important environmental factor regulating microbial activities such as phytoplankton (Hamre et al. 2008), bacterial production (Ogbebo & Ochs 2008) and viral growth (Fuhrman & Noble 1995). UVR can affect microbial organisms by damaging DNA (Buma et al. 2001), reducing or increasing the availability of dissolved organic matter (DOM) and nutrients (Ziegler & Benner 2000), and inhibiting larger grazers (Medina-Sánchez et al. 2006).

Effects of UVR on phytoplankton have received more attention earlier than those on viruses and bacteria. A large number of recent studies indicate that phytoplankton communities were inhibited by solar UVR from polar to tropical waters (Estevez et al. 2001, Gao et al. 2007). Aquatic bacteria have been shown to be sensitive to sunlight radiation, especially to the shortest-wavelength fraction of UV radiation (Müller-Niklas et al. 1995, Joux et al. 1999, Winter et al. 2001). Bacteria have previously been assumed to be more susceptible to UVR stress than larger eukaryotic organisms (e.g. phytoplankton) because

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of their smaller size and lack of UVR-screening pigments (Garcia-Pichel 1994, Lindell et al. 1995, Jeffrey et al. 2000). Bacteria are considered to be too small to develop effixcient photoprotection against UVR (Garcia-Pichel 1994) because their genetic material comprises a significant portion of their cellular volume (Jeffrey et al. 2000). Therefore, bacteria are probably among the most susceptible group to photodamage within the plankton (Garcia-Pichel 1994, Conan et al. 2008). On the other hand, opposite results showed that UVB indirectly stimulates bacterial growth by photodegrading DOM to low molecular weight compounds (Lindell et al. 1995, Obernosterer et al. 1999). In contrast to those studies on bacteria, previous studies on viruses reached a clear consensus that UVR is the main cause of viral destruction (Fuhrman & Noble 1995), which may indirectly affect bacterial community structure and dissolved organic carbon (DOC) in marine ecosystems by causing the release of the host cell's contents (Middelboe & Lyck 2002).

Most studies have focused on UVR effects on a single trophic level, but the effects of UVR are actually on the entire planktonic community. Relatively little information on the impact of solar UVR on species composition within natural ecosystems or on the interaction of organisms between trophic levels is available (Häder et al. 2011). Studies on UVR effects on a single trophic level can not be used to predict UVR impacts on the whole natural planktonic community structure (Bothwell et al. 1994). The shifts in the dominant microbial assemblages because of the variation in UVR exposure may modulate carbon allocation in the microbial loop. For example, the shifts in the balance of bacterial respiration and phytoplankton production likely determine whether there is a CO_2 sink or release in a marine ecosystem (Conan et al. 1999). Intensive debate has occurred on whether oligotrophic marine systems are autotrophic or heterotrophic (Dortch & Packard 1989). Little attention has been paid to whether UVR regulates autotrophic or heterotrophic processes. Thus, the simultaneous study of UVR effects on both phytoplankton and bacteria is helpful to understand how UVR changes the microbial structure and carbon allocation by promoting tolerant species and reducing sensitive ones.

The South China Sea is the second largest inland sea and is connected to the Pacific Ocean by the Luzon Strait between Taiwan and the Philippines. Solar radiation including UVR in the South China Sea is stronger than that in temperate and polar zones because of the low zenith angles and low ozone concentrations in this area (Madronich 1993); hence the South China Sea is an ideal location to explore the effects of high UVR on the microbial assemblages. Previous studies have shown that ultraviolet-B (UVB) inhibition on phytoplankton photosynthesis in the South China Sea was ~10 to 24% in the whole water column in coastal waters on sunny days (Helbling et al. 2003, Gao et al. 2007). However, UVR effects on other microbial assemblages (such as bacteria and viruses) are still unknown in the South China Sea, let alone the comparison of their sensitivities to UVR.

Our main objectives were to (1) compare primary production (PP), bacterial production (BP) and viral decay rates (VDR) with full solar radiation, i.e. photosynthetically active radiation (PAR) and UVR, and only PAR (without UVR) and (2) compare the sensitivities of bacteria, phytoplankton and viruses to UVR. These objectives are important in assessing the possible impacts of UVR on biogeochemical processes in the coastal and offshore oligotrophic subtropical South China Sea.

MATERIALS AND METHODS

Sampling and experimental setup

A cruise was conducted on the RV 'Shiyan II' of the South China Sea Institute of Oceanology during September 5 to 22, 2005, stopping at 9 stations located in the northern South China Sea (Fig. 1). Temperature and salinity vertical profiles were determined with a YSI® 6600 CTD sensor. After seawater was dispersed into 1 l Whirl-Pak bags with high UVR transmission, large zooplankton were removed using a 200 µm mesh net. These Whirl-Pak bags allowed 67% transmittance (averaged over wavelengths of 290 to 320 nm) of UVB, 75% transmittance of UVA (320 to 400 nm) and 95% transmittance of PAR (400 to 700 nm) (Noble & Fuhrman 1997).

Water samples were collected from depths of 100, 50, 10 and 0% of surface full solar radiation and were incubated under approximately the same light conditions from where they were obtained by using different neutral density screening. Incubations were conducted for 5 h from ~10:00 to ~15:00 h for the measurements of BP, PP and VDR with UVR + PAR (full solar radiation) and without UVR (PAR treatments). UVR transmittance of wavelengths <400 nm was blocked by covering the sample with Melinex film (DuPont Products). These Whirl-Pak bags covered with Melinex (PAR treatments) were exposed to

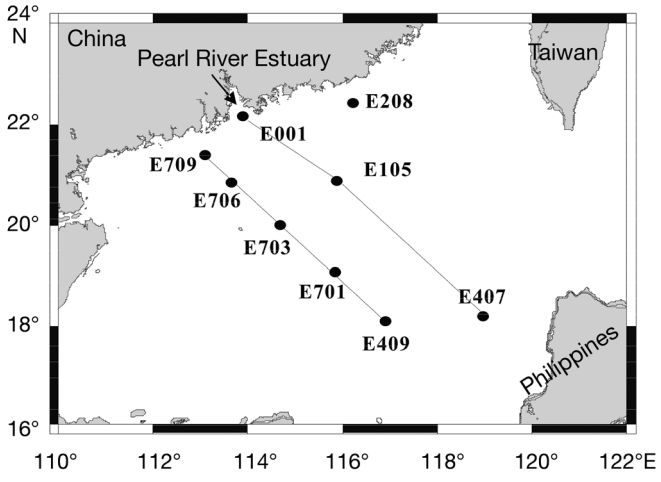

Fig. 1. Study area showing the locations of (black circles) sampling stations along 2 transects which were conducted from coastal (Stn E001 in the Pearl River estuary and Stn E709) to offshore waters (Stns E407 and E409) in the northern South China Sea (SCS) during September 2005. An incubation experiment was also conducted at Stn E208

solar radiation and incubated in a deck incubator cooled by surface seawater that did not capture the small temperature difference between deep (22 to 26°C) and surface (27 to 29°C) water.

The conditions of our experimental manipulation had some deviation from *in situ* natural seawater. The ratio of UVR/PAR under neutral density screens would be different from that of the *in situ* natural seawater because the light attenuation coefficient is higher for UVR than PAR in seawater.

Measurements of solar radiation

A vertical profile of solar radiation was measured with an IL 1700 radiometer (International Light) and with 3 different submersible, broad-band, photodiode sensors (UVA, UVB and PAR).

Measurements of biological parameters

Chlorophyll *a* (chl *a*) fluorescence was measured at the surface with a YSI® 6600 sensor and chl *a* of experimental water samples was measured using the *in vitro* fluorometric method with acetone extraction on a Turner Designs TD700 fluorometer (Knap et al. 1994).

For primary production, 10 to 20 µCi $NaH^{14}CO_3$ (14.8 to 74 kBq) was added in dim light to seawater (50 ml) from depths of 100, 50, 10 and 0% of surface light and incubated in Whirl-Pak bags for 5 h under full solar radiation (PAR + UVR) and PAR only. Triplicate bags were wrapped with different layers of screening that provided light fields corresponding to approximately 100, 50, 10 and 0% (i.e. darkness) of surface irradiance. The dark bottle for the control was wrapped in aluminum foil and placed in a black cloth bag. The incubation was terminated by filtering through a 25 mm Whatman GF/F filter and the filters were kept frozen (–20°C) until they were analyzed within 1 mo following the Joint Global Ocean Flux Study (JGOFS) protocols (Knap et al. 1994). The filters were put into scintillation vials containing 0.2 ml of 0.5 N HCl for 12 h in order to remove inorganic carbon. After the addition of 10 ml of scintillation cocktail (Hi-Safe) to each vial, samples were counted on a Perkin-Elmer Wallac 1414 liquid scintillation counter. Ambient dissolved inorganic carbon (DIC) was measured with an infra-red (IR) detector (Li-Cor 6252) using a DIC analyzer (AS-C2, Apollo SciTech), and bicarbonate incorporation rate was converted to µg C l^{-1} h^{-1} according to Knap et al. (1994). The use of GF/F filters may result in the underestimation of the primary production, as *Prochlorococcus* in oceanic waters might not be retained on GF/F filters efficiently.

Bacterial abundance (BA) was determined using the 4′,6-diamidino-2-phenylindole (DAPI) direct count method (Porter & Feig 1980). Samples (2 ml) were collected in micro-centrifuge tubes (Axygen) from separate Whirl-Pak bags, fixed with 2% (final concentration) formaldehyde, and filtered onto a 0.22 µm polycarbonate membrane filter (Poretics). Bacterial cells were observed with an epifluorescence microscope (Olympus BX41) with blue excitation (485 nm) and recorded as digital images. Stained bacterial cells in the digital images were acquired and counted with Image Pro® Plus (MediaCybernetics) software.

BP was determined as described by Simon & Azam (1989). The subsamples were collected into 2 ml micro-centrifuge tubes (Axygen) from separate Whirl-Pak bags after a 5 h incubation and incubated for 1 h in the dark to determine leucine incorporation. ^{3}H-leucine (final concentration 30 nmol l^{-1}; specific activity 55.9 Ci $mmol^{-1}$) was added to 2 ml subsamples (triplicate) with 1 control fixed by 5% tri-chloroacetatic acid (TCA), and the linearity of the incorporation of leucine was tested in a separate time series experiment (data not shown). The incubation was terminated by adding TCA (5% final concentration). After centrifugation and aspiration of the supernatant, pellets were rinsed and centrifuged twice with 1 ml of 5% TCA, and then scintillation cocktail was added to the

vial. The incorporated ^3H was determined using a Perkin-Elmer Wallac 1414 liquid scintillation counter. Conversion factors (CFs) for estimating cell production from rates of leucine incorporated were empirically derived from dilution culture experiments (Hoch & Kirchman 1993). Two experiments were conducted to determine the *in situ* CF (data not shown), and the estimated CFs were ~2 ± 1.2 kg C mol leucine^{-1}.

Viral samples were fixed with glutaraldehyde (2.5% final concentration) and stored in liquid nitrogen. The samples for enumeration of viruses were thawed immediately before analysis, diluted 10- to 100-fold in tris-EDTA (TE) buffer (pH 8) and stained with SYBR green I (Molecular Probes) at 80°C in the dark for 10 min. Fluorescent microspheres (Molecular Probes) with a diameter of 1 µm were added to all samples as an internal standard. The discriminator was set on green fluorescence, and the samples were analyzed at a viral event rate of between 100 and 1000 s^{-1}. Viral abundance was determined using flow cytometry (FACSCalibur, Becton Dickinson) according to methods described by Brussaard (2004). VDR was calculated as the percent decrease of viral abundance divided by the incubation period and assessed according to the method of Noble & Fuhrman (1997). In detail, 20 to 100 ml water samples were filtered (0.2 µm pore-size) and the filtrates were incubated for 6 h under full solar radiation and PAR only treatments. Subsamples (2 ml) were taken every 1 to 2 h and processed for viral counts using flow cytometry. From the decrease in viral abundance over time, the viral decay rates were calculated by fitting a linear regression of the logged viral abundance to time. The slope of the line is the VDR (h^{-1}).

Calculations and statistics

UVA diffuse attenuation coefficient (K_d) was calculated according to the equation: $E_Z = E_0 \exp(-K_d \times Z)$ where E_Z and E_0 are downwelling irradiances at depths Z and 0, respectively (Kirk 1994). Percent UVR inhibition of PP = $(PP_{PAR} - PP_{full}) / PP_{PAR}$, where PP_{PAR} is PP under PAR only and PP_{full} is PP under full solar radiation. The estimates of inhibition of UV on BP are similar to those on PP (see equation above). The significance of spatial differences was assessed using an analysis of variance followed by a means comparison (*t*-test) or 1-way ANOVA test at a significance level of p = 0.05. The error bars represent a pooled sample standard deviation of the means. In addition, the Pearson chi-squared test was used to obtain the correlation coefficient. All statistical analyses were performed using SPSS statistical software (IBM).

RESULTS

Environmental parameters of the study area

Daily solar radiation was relatively strong (1500 to 2100 J cm^{-2}) and higher than the average daily solar radiation (1400 J cm^{-2}) for the whole year in 2005 (www.weather.gov.hk). The UVR dose (UVA + UVB) varied from 70 to 120 J cm^{-2} during the incubation period (Fig. 2). UVA diffuse attenuation coefficients generally decreased from Stns E001 to E407, which significantly correlated with salinity ($r^2 = 0.9$, $p < 0.05$). UVA attenuation depths (1% of surface UVA) varied from 1.7 m at Stn E001 to 60 m at Stn E407 (Table 1). The lowest UVA penetration depth, 1.7 m at E001 (the highest attenuation coefficient, K_d, 2.7 m^{-1}) resulted from the influence of the freshwater discharge from the Pearl River estuary.

Salinity increased gradually from the Pearl River estuary (Stn E001) to offshore, ranging from 23 at coastal Stn E001 to 34 at offshore Stn E409 (Fig. 3). There were only small variations in surface temperature among all these stations, which ranged from 27°C at Stn E001 to 29°C at Stn E701 (Fig. 3).

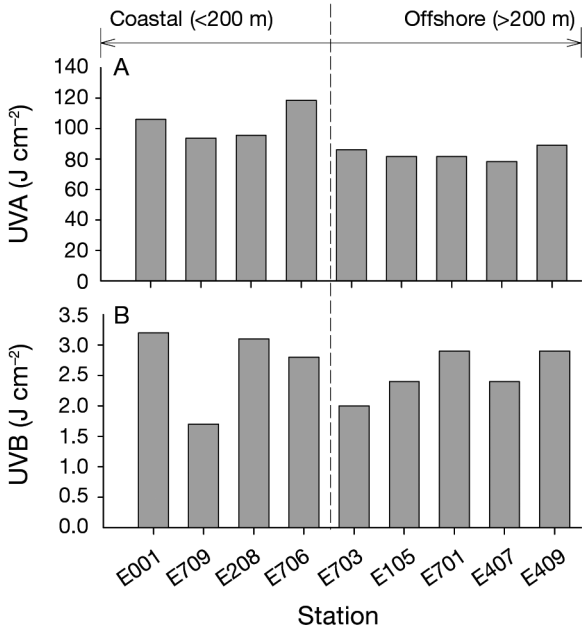

Fig. 2. Exposure doses of (A) UVA and (B) UVB at the surface at 9 stations in the South China Sea, shown in order of increasing salinity (see Table 1)

Table 1. Depth, temperature, salinity and chlorophyll *a* (chl *a*) in the surface water and diffuse attenuation coefficient of UVA (K_d) and attenuation depths of 1% surface UVA (Z_1) observed at 9 stations in the South China Sea. nd: no data. n = 1

Stns	Latitude (°E)	Longitude (°N)	Depth (m)	Temperature (°C)	Salinity	Chl *a* (µg l^{-1})	K_d (m^{-1})	Z_1 (m)
E001	22.00	114.00	20	27.9	23.5	3.0	2.70	1.7
E709	21.50	113.50	40	29.0	32.1	2.9	0.62	7
E208	22.20	116.30	40	29.4	33.2	0.2	0.33	14
E706	20.75	114.25	80	28.9	33.9	0.1	0.41	10
E703	19.90	115.10	1180	29.1	33.9	0.1	nd	nd
E105	20.60	116.40	450	29.1	34.0	0.1	0.24	20
E701	19.00	116.01	3100	29.1	34.0	0.3	0.11	40
E407	18.00	119.00	4150	28.1	34.1	0.1	0.08	60
E409	18.10	117.00	3950	27.7	34.2	0.1	0.12	40

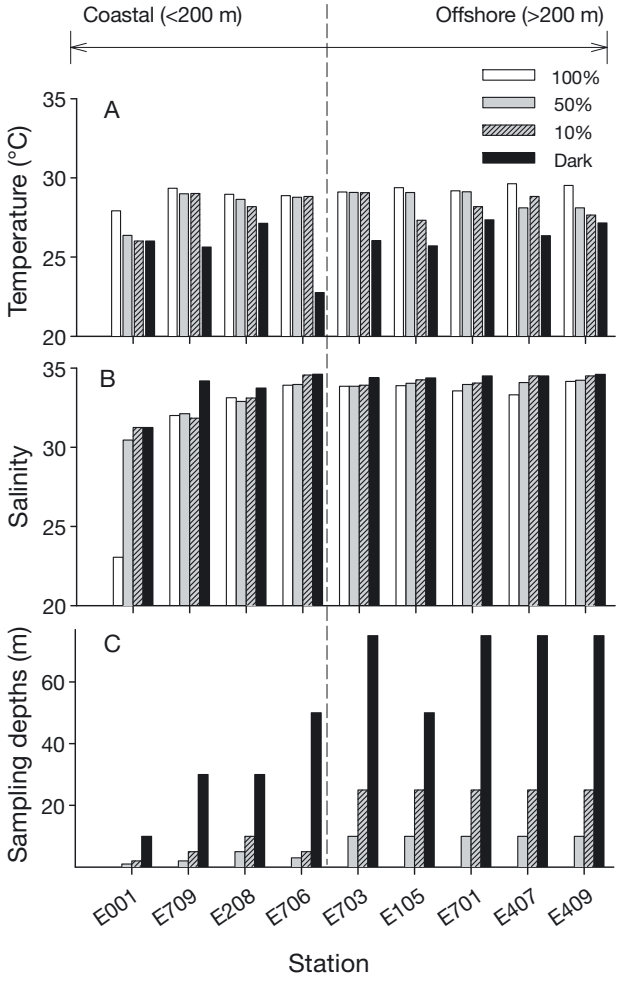

Fig. 3. (A) Temperature, (B) salinity, and (C) sampling depths corresponding to light depths of 100, 50, 10 and 0% (darkness) of full solar radiation (PAR + UVR) at 9 stations from coastal to offshore waters shown in order of increasing salinity. PAR: photosynthetically active radiation; UVR: ultraviolet radiation. n = 1

Biological parameters

Chl *a* and bacterial and viral abundances were often higher at coastal than offshore stations. Surface chl *a* was 3.0 µg l^{-1} at coastal Stn E001 and decreased to ~0.1 µg l^{-1} at offshore Stn E703 (Table 1). Surface bacterial and viral abundances were 7 to 14 × 10^5 cells ml^{-1} and 0.8 to 2 × 10^7 ml^{-1} in coastal waters, respectively, and 7 to 10 × 10^5 cells ml^{-1} and 2 to 10 × 10^7 ml^{-1} in offshore waters, respectively (Fig. 4).

Under full solar radiation (UVR + PAR), the highest surface PP was ~0.6 µg C l^{-1} h^{-1} at coastal Stn E001 and decreased to ~0.3 µg C l^{-1} h^{-1} in the offshore stations (Fig. 5A). Surface PP was significantly higher than that in subsurface samples in coastal and offshore waters except at Stns E208 and E409 (t = 2.6, df = 25, $p < 0.05$) (Fig. 5A).

The surface BP at Stn E001 was ~0.01 µg C l^{-1} h^{-1} and reached a maximum (~0.05 µg C l^{-1} h^{-1}) at Stn E709 (Fig. 5B). Surface BP was low (~0.02 µg C l^{-1} h^{-1}) at offshore Stns E701, E409 and E407. BP was often higher under 50% than 10% of full solar radiation at all these stations (t = 2.8, df = 13, $p < 0.05$) (Fig. 5B).

The coastal VDR at the surface were up to 0.1 h^{-1} at Stn E709, while VDR in the offshore waters were 0.01 to 0.11 h^{-1}. VDR was usually higher under 100, 50 and 10% of full solar radiation than in the dark (t = 2.1, df = 25, $p < 0.05$) (Fig. 5C).

UVR effects on PP, BP and VDR

Significant UVR inhibition of PP and BP was observed in most samples (Fig. 6A). UVR inhibition of PP varied from ~0 to 52% (Fig. 6A). Surface PP decreased by ~52% at Stn E001 because of the UVR exposure, which was the largest UVR inhibition of PP

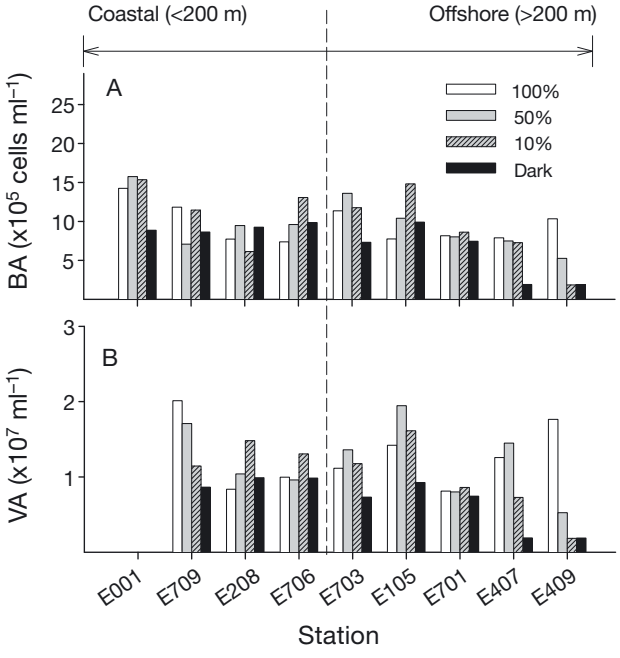

Fig. 4. (A) *In situ* bacterial abundance (BA) and (B) viral abundance (VA) under light depths of 100, 50, 10 and 0% (darkness) of full solar radiation (PAR + UVR, see Fig. 3 for definition) at 9 stations from coastal to offshore waters shown in order of increasing salinity. VA was not measured at Stn E001. n = 1

among all the measurements during this cruise (Fig. 6). The maximum inhibition of PP was not always at the surface, even though surface samples received a higher UVR dose (Fig. 6A). UVR inhibition of PP exhibited no correlation with UVA diffuse attenuation coefficient as well as with UVR dose at the surface (data not shown).

Due to the UVR exposure, the inhibition of BP was highest at E001 and was not always higher at the surface than the subsurface (Fig. 6B). At coastal Stn E208, BP was not inhibited by UVR at 50% of full solar radiation but increased by approx. 10% (negative inhibitory value). UVR inhibition of BP had no correlation with UVR dose and diffuse attenuation coefficient (data not shown).

In contrast to the UVR inhibition of PP and BP, VDR increased under full solar radiation compared to the PAR treatment. VDR was highly variable, ranging from ~0 to 120% (Fig. 6C). The percent increase in VDR was significantly correlated with the UVA diffuse attenuation coefficient at all light depths (r = 0.4–0.6, df = 9, $p < 0.05$) (Fig. 7). In addition, the slope of this regression was steeper in subsurface water (–126) (Fig. 7B) than surface water (–49) (Fig. 7A), suggesting that subsurface viruses were more influenced by the variations in the diffuse attenuation coefficient than surface viruses.

Comparison of UVR effects between PP, BP and VDR

A total of 20 out of 26 data points were above the 1:1 line for the inhibition of BP versus PP, indicating that BP was inhibited more than PP (t = 2.3, df = 25, $p < 0.05$) (Fig. 8A). Similarly, the loss of viral abundance was often higher than the inhibition of PP because of the UVR exposure in 16 out of 24 samples, although not significantly higher (t = 1.8, df = 25,

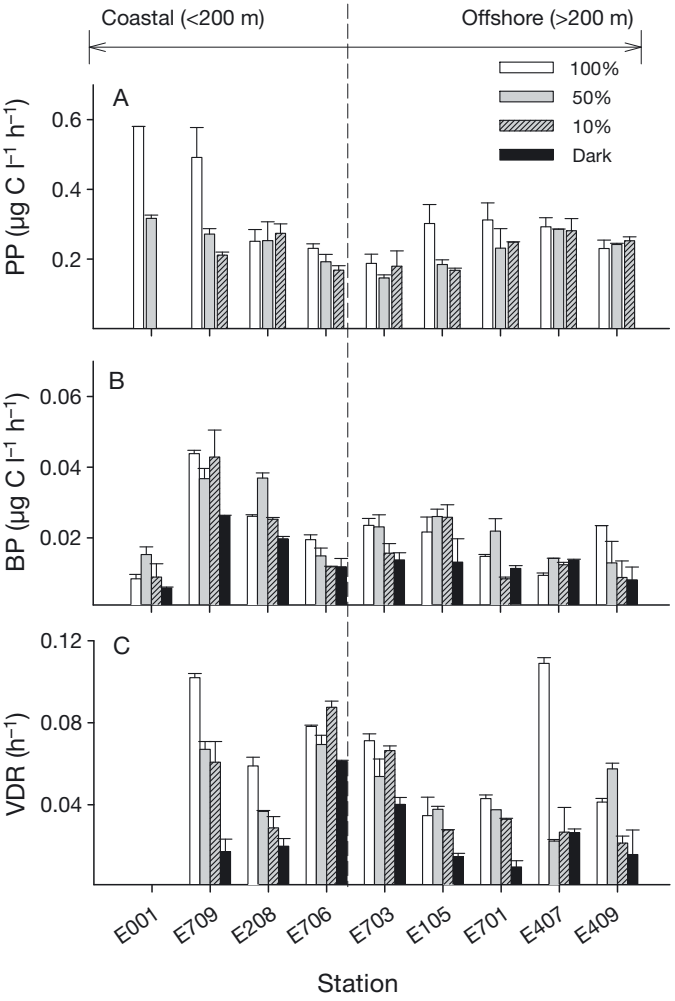

Fig. 5. Mean (± 1 SD) (A) primary production (PP), (B) bacterial production (BP), and (C) viral decay rates (VDR) under 100, 50, 10 and 0% (darkness) light depths for full solar radiation (PAR + UVR, see Fig. 3 for definition) at 9 stations from coastal to offshore waters shown in order of increasing salinity. At station E001, PP in 10% light depth was not measured because of shallow depth, and VDR was not measured. n = 3

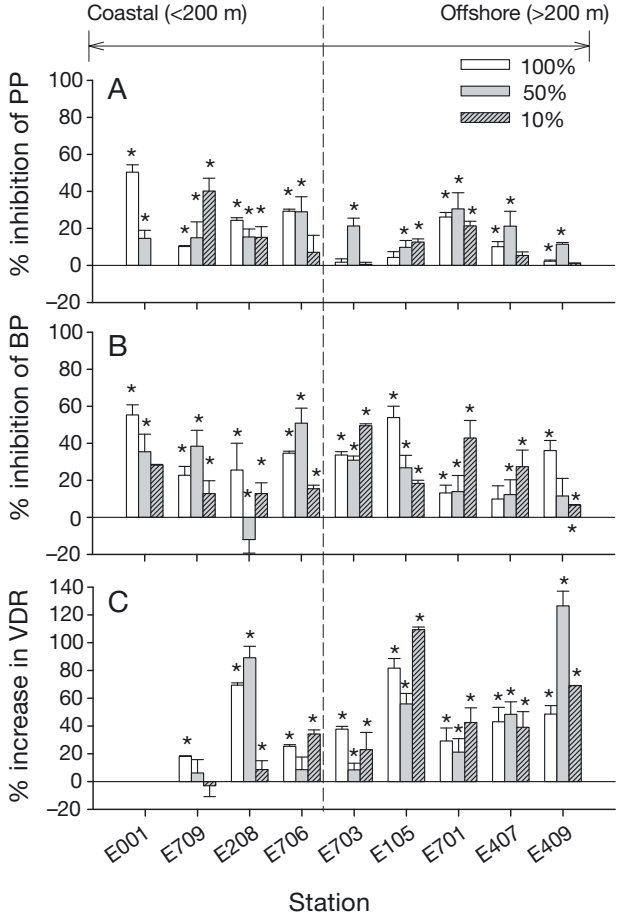

Fig. 6. Mean (± 1 SD) percent UVR inhibition of (A) primary production (PP), (B) bacterial production (BP) and (C) % increase in viral decay rates (VDR) under light depths of 100, 50, 10% of full solar radiation (PAR + UVR, see Fig. 3 for definition). Note that the scale on the *y*-axis is different between (B) and (C). UVR inhibition of VDR was not measured at Stn E001. n = 3. *p < 0.05 between samples under full solar radiation and PAR treatments. At station E001, PP in 10% light depth was not measured because of shallow depth, and VDR was not measured

p < 0.05) (Fig. 8B). However, UVR effects on VDR, BP and PP were not significantly higher under 100% than 10% and 50% of full solar radiation (t = 1.7, df = 26, p < 0.05) (Fig. 8A,B).

BP was significantly correlated with PP under UVR + PAR and in the PAR treatment (Fig. 9A,B). The test for homogeneity of the regression of BP versus PP showed that the slopes and the correlation coefficients were not significantly higher in PAR treatment than under full solar radiation (Fig. 9).

DISCUSSION

Factors regulating UVR effects

In offshore waters, UVR inhibition of PP, BP and the increase in VDR due to UVR was not always lower under 10 and 50% of full solar radiation than under 100% (t = 1.8, df = 9, p < 0.05). UVR inhibition might be higher in our incubation than the natural condition, especially at lower irradiances, because the experimental manipulation using neutral density filters would enhance the proportion of solar radiation with shorter wavelengths. Hence, the inhibition of BP, PP and the increase in VDR due to UVR was not always significantly correlated with UVR dose in our study (r = 0.1, p > 0.05, n = 20 to 24, data not shown).

UVR effects on these microbes did not only depend on the UVR dose (Cullen & Lesser 1991), but also was influenced by taxonomic composition, physiological status, light history, repair mechanisms and genetic acclimation (Helbling et al. 2003). For example, *Prochlorococcus* dominated the more oceanic and oligotrophic waters in the northern South China Sea, whereas *Synechococcus* was more important in mesotrophic areas (eddies, offshore jet and river

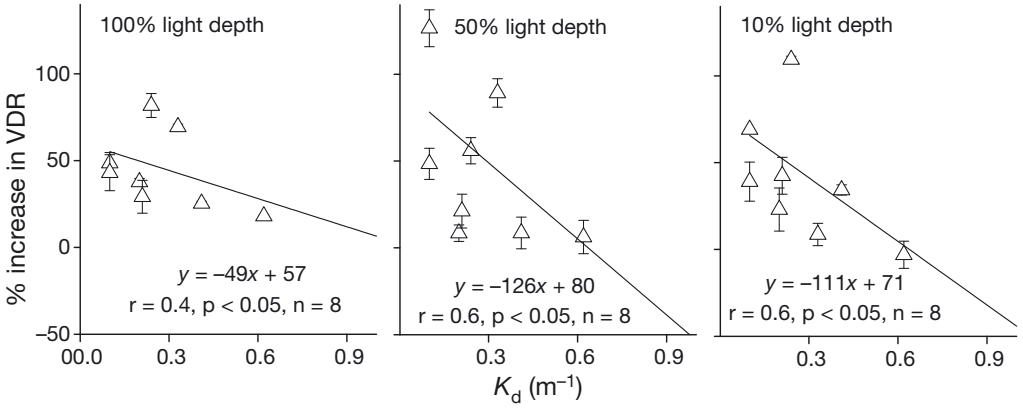

Fig. 7. Correlations of diffuse attenuation coefficient of ultraviolet radiation (K_d) with mean (±1 SD) viral decay rates (VDR) under 100, 50 and 10% of surface solar radiation. n = 8

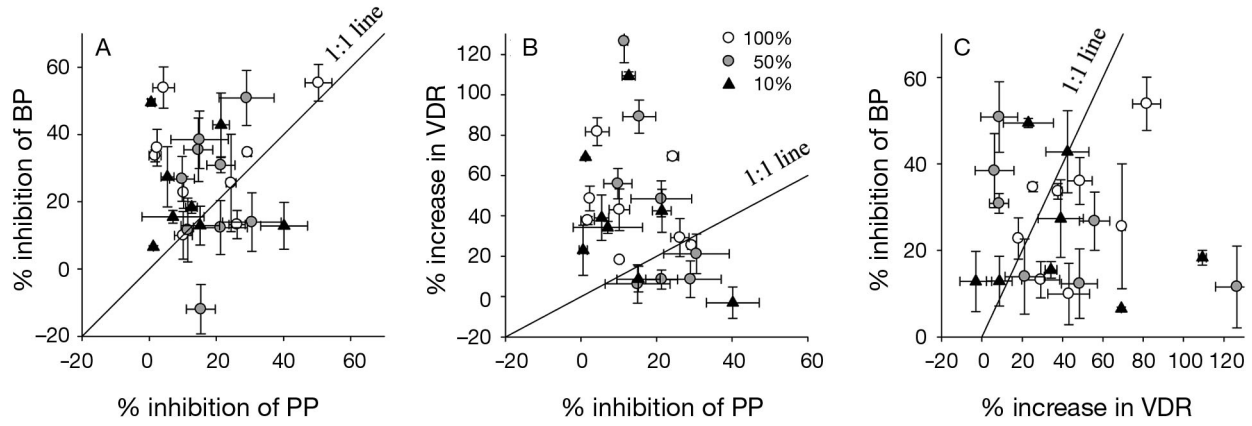

Fig. 8. Comparisons of ultraviolet radiation (UVR) effects on mean (±1 SD) bacterial production (BP), primary production (PP) and viral decay rate (VDR) at all 9 stations and 3 light depths (100, 50 and 10% of full surface radiation). (A) Inhibition of bacterial production (%) versus inhibition of primary production (%). (B) Increase in viral decay rate (%) versus inhibition of primary production. (C) Inhibition of bacterial production (%) versus increase in viral decay rate (%). Solid lines represent the 1:1 ratio. n = 24 to 27

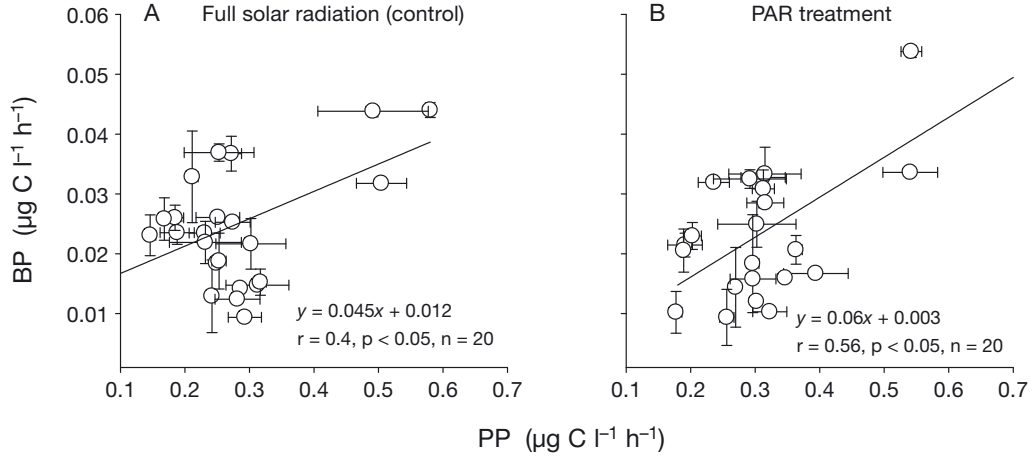

Fig. 9. Regression plots of mean (±1 SD) primary production (PP) versus mean (±1 SD) bacterial production (BP) under (A) full solar radiation (UVR + PAR, see Fig. 3 for definition) and (B) PAR only treatments. n = 24 to 27

plume) (Chen et al. 2009). The distribution patterns of these picoplankton may affect UVR inhibition, as some studies have reported that *Synechococcus* and eukaryotes were more resistant to UVR than *Prochlorococcus* in oligotrophic waters (Llabrés & Agustí 2006, Agustí & Llabrés 2007). However, our study showed that UVR inhibition of PP was not significantly higher in oceanic and oligotrophic waters than coastal and estuarine waters ($t = 1.1$, df = 28, $p < 0.05$) (Fig. 6A).

Vertical mixing often influenced the UVR effects on microorganisms, and deeper phytoplankton were less resistant to UVR than surface cells because of their different light history (Helbling et al. 2003). Vertical mixing such as upwelling and downwelling was likely an important factor regulating the microbial sensitivities to UVR in the South China Sea. During the same cruise, He et al. (2009) observed that upwelled cold and saline water was present at Stn E001 and downwelling was present at Stn E105. In our study, UVR inhibition of PP at Stn E001 was higher than that at Stn E105 ($t = 3.1$, df = 4, $p < 0.05$) even though the inhibition of PP was normalized in terms of UVR dose (data not shown). Hence, surface phytoplankton in upwelled waters (Stn E001) were more sensitive to UVR exposure possibly because of their previous light history in deeper waters ($t = 3.4$, df = 4, $p < 0.05$) (Fig. 6A). Similarly, previous studies

also reported that bacteria in surface water samples showed a more effective photorepair mechanism than subsurface assemblages in the upwelling system off central–southern Chile (Hernández et al. 2006). However, in our study, UVR inhibition of surface BP was not significantly different between Stns E001 and E105 (t = 0.8, df = 4, $p > 0.05$) (Fig. 6B). Hence, surface bacteria in upwelled and downwelled waters did not exhibit a difference in resistance to UVR in our study, although vertical mixing has been shown to influence the DNA damage and viability of bacteria (Jeffrey et al. 1996, Hernández et al. 2006, Bertoni et al. 2011).

The freshwater discharge is characterized by heavy organic loads in the Pearl River estuary, which influences its optical properties (Wang et al. 2010). As a result, the UVA diffuse attenuation coefficient was high at Stn E001 and adjacent waters (Table 1). Hence, although the UVR dose at the surface was not considerably different during our incubations at 9 stations, UVR penetration was quite different between coastal and offshore waters and was much lower near the Pearl River estuary (Table 1). There was a significant correlation between VDR and diffuse attenuation coefficient, and VDR were higher in offshore waters (Fig. 7C) where there was higher light penetration. However, neither BP nor PP was significantly correlated with the diffuse attenuation coefficient (data not shown). Hence, viruses appeared to be more affected by light attenuation than phytoplankton and bacteria possibly because the waters with high light penetration provided less protection for viruses from the UVR exposure. In addition, viruses have no repair mechanism, although Weinbauer et al. (1997) reported that light-dependent repair, probably photoreactivation, compensated for a large fraction of sunlight-induced DNA damage in natural viral communities. Hence, viruses are more sensitive to variations in water optical properties than bacteria and phytoplankton.

In addition to optical properties, the Pearl River estuary also influenced the chemical properties of the adjacent waters, especially the inorganic N:P ratios because the upstream freshwater from the Pearl River estuary exhibits a high N:P ratio (100:1), which often results in P-limitation for surface phytoplankton and bacteria (Yin et al. 2001, Yuan et al. 2011b,c). Both phytoplankton and bacteria were also strongly inhibited by UVR at Stn E001 (Fig. 6A,B), where N:P ratio was >30:1 as reported by He et al. (2009). Further studies are needed to determine if the dual stress of UVR and P-limitation near the Pearl River estuary makes the bacteria and phytoplankton less resistant to UVR effects (Fig. 6). Previous studies showed that P-limited phytoplankton were often less resistant to UVR inhibitory effects (Doyle et al. 2005). Medina-Sánchez et al. (2002) also reported that UVB radiation inhibited bacteria when they were strongly P-deficient, whereas UVB exerted no direct effect on bacterial activity when they were not P-limited. A recent study showed that phosphorus amendment and UVR exposure had a synergistic negative effect on phytoplankton (Carrillo et al. 2008).

UVR effects on viral decay rates (VDR)

While ~40 ± 35% of viral mortality was due to UVR effects at the surface in the South China Sea, VDR was relatively low (0.01 to 0.11 h^{-1}) (Fig. 6) in comparison with high VDR of 0.4 to 0.8 h^{-1} in the Gulf of Mexico (Wilhelm et al. 1998) and 0.05 to 0.1 h^{-1} in Santa Monica Bay (Noble & Fuhrman 1997). The low VDR might be due to the fact that viruses from sunnier regions apparently show more adaptation and resistance to light damage (Noble & Fuhrman 1997). However, because of the sparse historical data on the UVR contribution to VDR in other offshore regions, the comparison of viral sensitivity to UVR in the South China Sea with other oligotrophic offshore systems requires further investigation based on the same UVR dose and spectra during the same month.

Viruses play an important role in the biogeochemical cycling of carbon and nitrogen in the ocean (Fuhrman 1999, Suttle 2005, 2007) and can be modulated by variations in UVR dose. Assuming that all viruses were bacteriophages because algal viruses do not significantly contribute to total viral abundance (Wommack & Colwell 2000), the viral contribution to bacterial mortality is equal to [VDR × viral abundance / (burst sizes × bacterial abundance)] (Culley & Welschmeyer 2002). A range of burst sizes between 50 and 100 viruses for one bacterial cell was used in our calculations (Bratbak et al. 1990, Wilhelm et al. 2002). Because surface VDR varied from ~0.01 to 0.11 h^{-1}, surface viral activity could contribute to about 0.4 to 1% h^{-1} of the surface bacterial mortality. If bacterial biomass (C) was estimated using a CF of 30 and 12 fg C $cell^{-1}$ for coastal and offshore waters, respectively (Fukuda et al. 1998), the surface bacterial mortality because of viral lysis was approximately equal to 20 to 50% BP. About 30% of the viral mortality was due to UVR exposure at the surface, which partially reduced the viral effect on bacteria and hence indirectly reduced BP mortality.

Implications for UV effects on trophic levels

Vidussi et al. (2011) reported that increased UVB only exerted moderate and insignificant effects on the entire plankton food web because of the moderate natural daily UVB dose combined with a relatively UVB-resistant plankton community in early spring in the northern Mediterranean. In contrast to their moderate effects, our results showed that strong UVR inhibited phytoplankton, bacteria and viruses, as our experiments were conducted under the whole UVR spectra rather than UVB only. In agreement with the study by Plante & Arts (2000), which showed competitive advantages of phytoplankton over bacteria under UVR in continuous cultures, our results also showed that bacteria were more inhibited by UVR than phytoplankton were.

However, the UVR effects on BP and PP were not directly comparable in our study. In contrast to UVR effects on VDR and PP, which were estimated based on accumulative effects over a 5 h exposure, the effects of UVR on BP were measured with ^{3}H-leucine within 1 h in the dark after a 5 h exposure to UVR. Because bacteria can partially recover from UVR damage in the dark (Jeffrey et al. 1996), the inhibition of UVR on BP would be expected to be higher if BP was measured similar to PP by incubating the sample with radiolabeled substrates during the whole 5 h exposure. In addition, Vaughan et al. (2010) also showed that inhibition of UVR on BP was less when radiolabeled substrates were added after exposure to UVR, compared to simultaneous exposure and incorporation. Therefore, the UVR effects on BP might be underestimated in our study because BP was measured with ^{3}H-leucine in the dark at the end of the exposure. However, if there is an underestimate, then our results even more strongly support our conclusion that UVR inhibition of BP was significantly higher than PP in most of the samples.

The slope and correlation coefficients between BP versus PP were lower under full solar radiation than under the PAR treatment (Fig. 9), suggesting that the correlation between BP and PP became weakened because of UVR. Several previous studies also suggested that the phytoplankton–bacteria relationship can be controlled by UVR based on the following evidence. (1) UVR exerted a negative effect on primary production but strongly enhanced the absolute and percentage excretion of C (up to 60%) (Carrillo et al. 2002). The availability of this excreted carbon for bacteria requires further study. (2) Bacteria may be indirectly affected by UVR through the trophic cascade. For example, UVR decreased the bacterivorous capability of nanoplankton and consequently reduced the predators on bacteria (Medina-Sánchez et al. 2006). In addition, phytoplankton would exhibit different responses in different nutrient states. For example, nutrient stress may weaken or mask the UVB stress (Xenopoulos et al. 2002), while a nutrient amendment (e.g. P addition) had a negative effect on phytoplankton subjected to UVR exposure (Carrillo et al. 2008).

An important question concerning the marine ecosystem functionality is whether it is autotrophic or heterotrophic, although the trophic state does not always determine whether the water is a source or sink of CO_2 (Chen 2010, Yuan et al. 2011a). Bacterial respiration has a large contribution to the release of CO_2 in coastal waters of the South China Sea (Yuan et al. 2010), while high phytoplankton production tends to enhance the biological pump by CO_2 drawdown (Carlson et al. 1994). However, our study of BP was not sufficient to estimate the effects of UVR on heterotrophic activities because a large part of the bacterial carbon demand is respired and bacterial respiration may not be inhibited in the same way as BP. In addition, BP measured after a 5 h incubation would not represent the activities during the whole day because bacteria are able to repair their UV damage during the night. Therefore, net community production (NCP), which includes the activities of all other organisms (e.g. zooplankton), would be a more useful parameter to determine in order to understand the effects of UVR on trophic state. For example, NCP was severely inhibited by UVR at the surface in mesotrophic low salinity waters adjacent the Rhône River (Joux et al. 2009). Therefore, further experiments on NCP are needed to determine UVR effects on heterotrophy or autotrophy in the South China Sea.

In summary, the sub-tropical/tropical South China Sea is characterized by strong solar radiation and high UVR especially in summer. Hence it is important to determine strong UVR effects on the food web structure and the consequences on carbon flow. Our results showed that the effects of strong UVR on phytoplankton, bacteria and viruses were generally inhibitory in late summer in the northern South China Sea. UVR exposure decreased viral abundance, BP and PP by 30, 20 and 14%, respectively. The inhibitory effects on phytoplankton, bacteria and viruses depended on the water optical and chemical properties, which were influenced by the Pearl River estuarine waters and the mixing with offshore waters. The comparison of phytoplankton and bacterial UVR inhibition indicated that there was a higher

decrease in BP than PP because of the UVR exposure (t = 2.3, df = 25, $p < 0.05$) (Figs. 5, 6 & 8). Therefore, UVR might shift a marine ecosystem to more phytoplankton dominance because of UVR exposure during the daytime.

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