Bacterial production and respiration in subtropical Hong Kong waters: influence of the Pearl River discharge and sewage effluent

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ABSTRACT: Hong Kong waters are influenced by the Pearl River discharge in the west, coastal/oceanic waters in the east, and year-round domestic sewage effluent in the Victoria Harbour area. Seven cruises were conducted at 12 stations across the Hong Kong water in the dry and wet seasons to examine how the Pearl River outflow and sewage effluent discharge influenced the distributions of dissolved oxygen (DO), dissolved inorganic carbon (DIC), bacterial production (BP) and bacterial respiration (BR). Surface DO saturation was usually ~80%, and surface partial pressure of carbon dioxide (pCO_2) averaged ~570 µatm at all 12 stations. The undersaturated DO and supersaturated pCO_2 indicated that Hong Kong waters were heterotrophic and a net source of CO_2 to the atmosphere, except during periods of phytoplankton blooms when pCO_2 became very low (<200 µatm). The Pearl River discharge in the wet season decreased DO and increased pCO_2 and BP in the estuarine-influenced waters in comparison with the coastal/oceanic waters. Sewage effluent exerted a strong influence on carbon dynamics and CO_2 efflux as surface pCO_2 was significantly correlated with NH_4, an indicator of sewage effluent. BR in Hong Kong waters was ~100 to 400 mmol C m^{-2} d^{-1} in July and November 2005. The BR fraction accounted for 50 to 80% of total dark community respiration (DCR) in coastal/oceanic waters with less eutrophic inputs, but increased to >90% of DCR in more eutrophic waters near the sewage discharge site, likely due to the decrease in phytoplankton biomass. Contribution of BR to the CO_2 efflux was ~3 to 10 mmol C m^{-2} d^{-1}, which was ~50% (varied from 16 to 130%) of the total CO_2 efflux. This study has important implications for severely anthropogenically impacted coastal areas, as they may be an important source of atmospheric CO_2 due to active BR.

KEY WORDS: Bacterial production · Bacterial respiration · CO_2 · Sewage effluent · Pearl River estuary

INTRODUCTION

Coastal and estuarine waters receive large amounts of inorganic nutrients as well as terrestrial organic matter from river and sewage effluent discharge. Eutrophication has become a major concern in these areas (NRC 2000), since eutrophication not only increases primary production (Lohrenz et al. 1999), but also increases high bacterial production (BP) and bac-

terial respiration (BR) (Del Giorgio et al. 1997, Choi et al. 2005) and consequently hypoxic events such as those observed in the Yangtze River plume (Li et al. 2002), the Gulf of Mexico (Rabalais et al. 1994), and the Loire estuary (Abril et al. 2003). In addition, high BR also results in a net CO_2 source (or CO_2 supersaturation), especially when there are high terrestrial inputs of organic matter in marine ecosystems (Smith & Hollibaugh 1993, Del Giorgio et al. 1997), as suggested by

studies in the East China Sea (Chen et al. 2003), Mississippi River plume (Chin-Leo & Benner 1992), northern North Sea during a coccolithophore bloom (Robinson et al. 2002b) and eutrophic lakes (Biddanda et al. 2001). However, it is generally unclear whether terrestrial and sewage inputs lead coastal waters to be more heterotrophic or autotrophic.

Recently, CO_2 supersaturation has been reported in a number of estuarine systems such as the York, Satilla, Scheldt and Pearl River estuaries (Raymond et al. 1997, Cai & Wang 1998, Frankignoulle et al. 1998, Dai et al. 2006). CO_2 super- or undersaturation provides qualitative evidence of net hetero- or autotrophy (Raymond & Bauer 2000). However, whether the global coastal oceans are net heterotrophic or autotrophic depends on the area and variations in many seasonal factors (Ducklow & McCallister 2004). Many studies of CO_2 variations focused on temperate coastal waters, in which CO_2 concentrations are variable (from 75% undersaturation to ~200% oversaturation), whereas the subtropical region has been less well studied (Ducklow & McCallister 2004, Cai et al. 2006). One recent study reported that coastal waters of the South China Sea (SCS) were dominated by oversaturated CO_2 (~400 to ~600 µatm) in July 2000, May 2001 and November 2002 (Zhai et al. 2005), but further studies are necessary with more monthly coverage. Hong Kong is situated on the northern side of the SCS, and hence our study of seasonal CO_2 variations provides further evidence of the heterotrophy or autotrophy in coastal waters of the subtropical SCS, and quantify the contribution of heterotrophic bacteria to the process.

As the population in southern China (including Hong Kong) continues to increase, the inorganic nutrient loading is also increasing through domestic and industrial sewage discharge in the Pearl River estuary (PRE) and adjacent waters. Apart from the increases in inorganic nutrients, the river discharge and sewage effluent may export organic matter into coastal waters, which could be oxidized by bacteria (Kirchman et al. 1991) and lead to an increase in partial pressure of carbon dioxide (pCO_2). The input of organic matter into coastal ecosystems alters biogeochemical cycling processes (e.g. nutrient and carbon cycling) in coastal waters. Previous studies in these areas suggest that these anthropogenic inputs of inorganic nutrients and organic matter have led to variations in phytoplankton biomass, oxygen and CO_2 in the PRE and adjacent waters (Yin et al. 2004, Cai et al. 2004, Dai et al. 2006). However, there is still little information about how these inputs from the PRE and sewage effluent affect heterotrophic bacteria and influence their rates of oxygen consumption and CO_2 release.

In 2005 and 2006 we conducted 7 cruises and visited 12 stations during each cruise to (1) assess whether the influence of the PRE and sewage effluent discharge resulted in net heterotrophy in Hong Kong waters; (2) investigate the variations in dissolved oxygen (DO), CO_2, and BP during dry and wet seasons; and (3) determine the contribution of bacterial respiration to oxygen consumption and CO_2 release in these coastal waters. This is a study on severely anthropogenically impacted subtropical coastal areas, which are an important source of atmospheric CO_2 due to BR.

MATERIALS AND METHODS

Study area. Hong Kong is situated on the eastern side of the PRE (Fig. 1). The annual average Pearl River discharge is 10 500 m^3 s^{-1}, with 20% occurring during the dry season from October to March and 80% during the wet season from April to September (estimated by Yin et al. 2000 from Zhao 1990). The PRE carries pollutants and high nutrient loadings due to local sewage and agricultural inputs (Cai et al. 2004, Yin et al. 2004). Hong Kong waters receive >2 000 000 t of sewage effluent daily from the local sewage discharge in Victoria Harbor (VH). In 2001 the Hong Kong government implemented the Harbor Area Treatment Scheme (HATS), which collects and treats 70% of the sewage that was previously discharged into VH.

Sampling. Sampling was conducted at 12 stations (Fig. 1) and 2 depths: (1) 1 to 4 m below the surface; and (2) 2 m above the bottom during 7 cruises in June, July and November 2005 and March and April, July and November 2006. Stns 1 to 12 correspond to the same stations used in the Hong Kong Environmental Protection Department's monitoring program (EPD, www.epd.gov.hk) (Table 1). Stns 1 and 2 represent the western waters. Stns 3, 4 and 5 are near the sewage discharge site in VH, and Stn 8 is considered to be a reference station, since it receives little anthropogenic input from the PRE or the local sewage discharge site. Stns 9 to 12 in the southern waters represent a eutrophic gradient from Stn 9 (~10 m) to Stn 12 (~35 m) in deeper coastal waters. The vertical profiles of salinity, temperature and chlorophyll *a* (chl *a*) were measured with a YSI® 6600 sensor.

Chl *a*, BA and BP. Chl *a* fluorescence was measured with a YSI® 6600 sensor, and chl *a* was also measured on discrete water samples using the *in vitro* fluorometric method with acetone extraction and measured on a Turner Designs TD-700 fluorometer (Knap et al. 1994).

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 microcentrifuge tubes (Axygen), fixed with
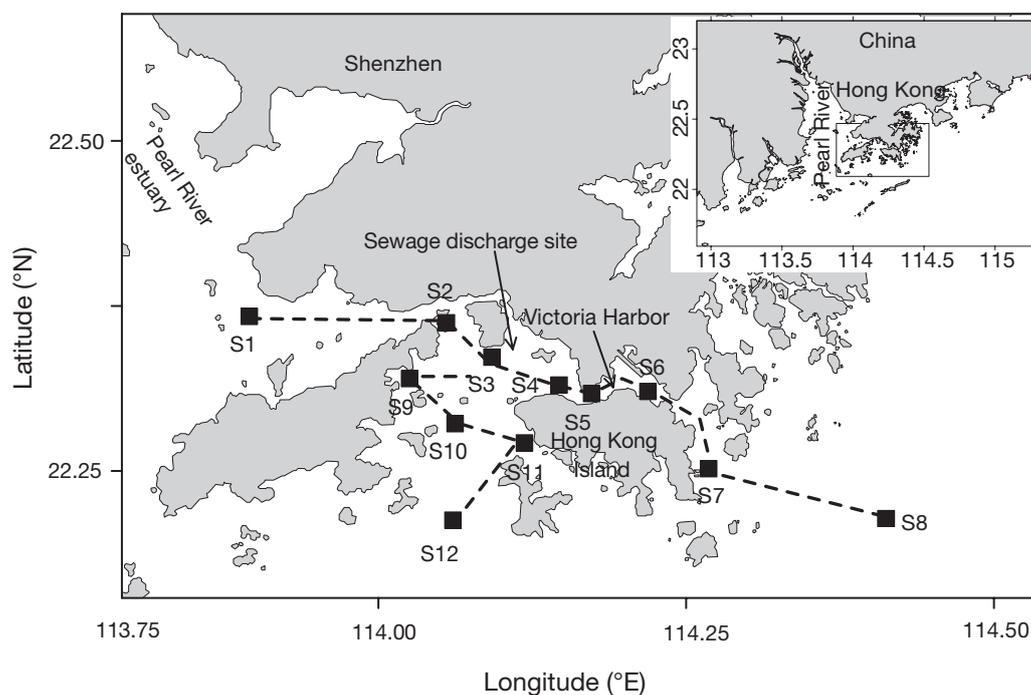
Fig. 1. Sampling stations along 2 transects (– – – –): a west to east transect from Stn 1 in estuarine waters to Stn 8 in eastern waters (EW), and a north to south transect from Stns 3 to 12. The new sewage discharge site is located between Stns 3 and 4

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 on digital images. Stained bacterial cells on the digital images were acquired and counted with Image Pro® Plus software (Media Cybernetics).

BP was determined as described by Simon & Azam (1989). ^{3}H-leucine (final concentration 30 nM, specific activity 55.9 Ci mmol^{-1}) was added to 2 ml subsamples (triplicate) with one control fixed by 5% trichloroacetic acid (TCA). The subsamples were incubated for leucine incorporation for 1 h, and the linearity of the incorporation of leucine was tested in a separate time series experiment (data not shown). The incubation

Table 1. Monthly average depths, temperature, salinity, dissolved oxygen (DO) and DO % saturation (DOs) (mean ± 1SD) at the surface in the wet and dry seasons during 2005 and 2006. Station numbers used by Environmental Protection Department (EPD) Hong Kong are also given

Stn	EPD	Depth (m)	Wet season				Dry season			
			Temp. (°C)	Salinity	DO (µM)	DOs (%)	Temp. (°C)	Salinity	DO (µM)	DOs (%)
1	NM2	12	28 ± 0.7	14 ± 7	194 ± 25	87 ± 13	24 ± 3	31 ± 1	209 ± 13	97 ± 4
2	WM4	32	27 ± 1	24 ± 4	178 ± 3	81 ± 2	23 ± 3	33 ± 0.6	203 ± 13	93 ± 3
3	WM3	19	26 ± 0.5	25 ± 4	169 ± 6	76 ± 3	23 ± 3	33 ± 0.3	206 ± 31	94 ± 9
4	VM7	11	26 ± 1	27 ± 4	169 ± 16	78 ± 7	23 ± 3	33 ± 0.3	184 ± 25	86 ± 5
5	VM5	13	26 ± 0.8	27 ± 4	181 ± 6	82 ± 3	23 ± 3	33 ± 0.3	163 ± 34	77 ± 9
6	VM2	14	26 ± 1.1	29 ± 3	209 ± 34	96 ± 16	23 ± 3	33 ± 0.3	181 ± 31	80 ± 10
7	EM3	25	25 ± 0.6	31 ± 1	225 ± 34	104 ± 18	23 ± 3	34 ± 0.3	219 ± 19	99 ± 6
8	MM8	31	26 ± 0.3	30 ± 2	253 ± 50	117 ± 23	25 ± 3	34 ± 0.3	225 ± 16	105 ± 10
9	SM10	6	28 ± 1	23 ± 5	272 ± 31	126 ± 14	23 ± 3	33 ± 1	222 ± 19	103 ± 10
10	SM9	9	27 ± 1	23 ± 6	172 ± 3	78 ± 2	23 ± 3	33 ± 1	206 ± 22	95 ± 5
11	WM1	33	28 ± 1	24 ± 5	191 ± 13	89 ± 6	23 ± 3	33 ± 0.5	206 ± 19	94 ± 3
12	SM6	15	28 ± 1	22 ± 6	250 ± 56	113 ± 26	23 ± 3	33 ± 0.5	216 ± 16	100 ± 2

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 ^{3}H was determined using a Perkinelmer Wallac, 1414 scintillation counter. Two experiments were conducted to determine the *in situ* conversion factors (CFs), and the estimated CFs were ~6 ± 4 and ~4.6 ± 2 kg C mol leucine^{-1} in the wet and dry season, respectively, assuming a cellular biomass of bacteria (20 fg C cell^{-1}). Bacterial growth efficiency (BGE) was also calculated using an empirical CF of ~3 kg C mol leucine^{-1} (Pedrós-Alió et al. 1999).

DO, DCR and BR. DO was determined in duplicate by Winkler titration, as outlined in the Joint Global Ocean Flux Study (JGOFS) protocols (Knap et al. 1994). DO solubility was calculated according to Benson & Krause (1984). After 4 to 5 ml of water was allowed to overflow from the 60 ml custom made BOD bottles when taking DO samples from the Niskin bottle, Winkler reagents were added. Winkler titrations were carried out in the laboratory with an automated titration apparatus (716 DMS Titrino, Metrohm®) that analyzed the samples with a potentiometric detector to determine the endpoint (Outdot et al. 1988).

In order to measure dark community respiration (DCR), samples in BOD bottles (60 ml) were incubated in a water circulation tank, simulating *in situ* temperature (±1.5°C). DCR was determined from changes in DO during 24 h incubation. The variation coefficient of DCR measurement was ~9 ± 5%, which was considered to be precise enough to measure respiration in coastal waters, although 5 replicates for respiration measurements have been recommended for oceanic waters where DO respiration is often <3 µM d^{-1} (Robinson et al. 2002).

BR was measured at Stns 3, 5, 7 and 12 in July and November 2005 using the DCR method described above. A total of 4 bottles for the initial and 4 bottles for the dark incubation were filled with the <1 µm size-fractionated seawater, which was gravity-filtered (or with a low pressure <15 cm Hg) onto 1 µm polycarbonate membrane to avoid breakage of fragile cells. The membrane had to be replaced frequently to prevent it from being blocked by particles.

BR (or DIC produced by bacteria) is presented in carbon unit assuming the respiratory quotient (RQ) to be 1. Contribution of BR to gaseous CO_2 efflux was calculated according to the increase in DIC due to BR, and then the increase in pCO_2, which was estimated by the equilibrium equation in the seawater (Mehrbach et al. 1973, Cai & Wang 1998) assuming alkalinity did not change. CO_2 flux was estimated using the stagnant-film model (Wanninkhof 1992).

DIC and pCO_2. Water samples (50 ml) were preserved with saturated $HgCl_2$ (20 µl) for DIC analysis and stored in a cool (4°C) dark chamber. DIC was measured within 1 wk by the subsequent quantification of CO_2 with an infrared radiation (IR) detector (LI-COR 6252). This method has a precision of 0.1 to 0.2% (Cai & Wang 1998). DIC was calibrated against certified reference material from A. Dickson, Scripps Institution of Oceanography, and a duplicate analysis was made every tenth sample (Dickson & Goyet 1994). pH was measured with an Orion ROSS combination glass electrode that was calibrated against 3 National Bureau of Standards (NBS) standards (Dickson & Goyet 1994). The precision of pH measurement was ~0.01. pCO_2 was calculated from measured pH values and DIC concentrations for estuarine and coastal waters (Cai & Wang 1998). Direct and indirect pCO_2 measurements have been previously determined to be in good agreement over a wide range of pCO_2 and salinity values (Frankignoulle & Borges 2001). In order to remove the temperature effect, pCO_2 was normalized to an annual average temperature, using the equation by Takahashi et al. (2002). The CO_2 flux across the air-sea interface is calculated by following the stagnant-film model (Wanninkhof 1992). The atmospheric pCO_2, which varies by ±20 µatm, was recorded to be ~370 µatm in the SCSwhich varies by ±20 µatm (Zhai et al. 2005).

Inorganic nutrients. Samples for nutrients were taken with a 60 ml syringe and filtered through a precombusted Whatman GF/F filter mounted in a Swinnex filter holder and dispersed into Nalgene bottles (Thermo Fisher Scientific). All plastic-ware was precleaned with 10% HCl. The filtered water samples were placed in a cooler with dry ice and frozen until analysis. Nutrients (nitrate, phosphate and silicate) were measured with a Skalar SAN autoanalyzer following Joint Global Ocean Flux Study (JGOFS) protocols (Knap et al. 1994).

Statistical analyses. The significance of seasonal and spatial differences was assessed using an analysis of variance followed by a means comparison (2-tailed *t*-test) or 1-way ANOVA test, and a significance level of 0.05 was used to determine a significant statistical difference. The error bars represent a pooled sample SD of the means. In addition, the Pearson chi-squared test was used to obtain the correlation coefficient.

RESULTS

Temperature and salinity

In the dry season, the average surface temperature was ~23 to 25°C at all stations (Table 1), while surface salinities varied from ~30 to 34, indicating the dominance of coastal seawater at all stations in Hong Kong waters. In the summer wet season, temperature was
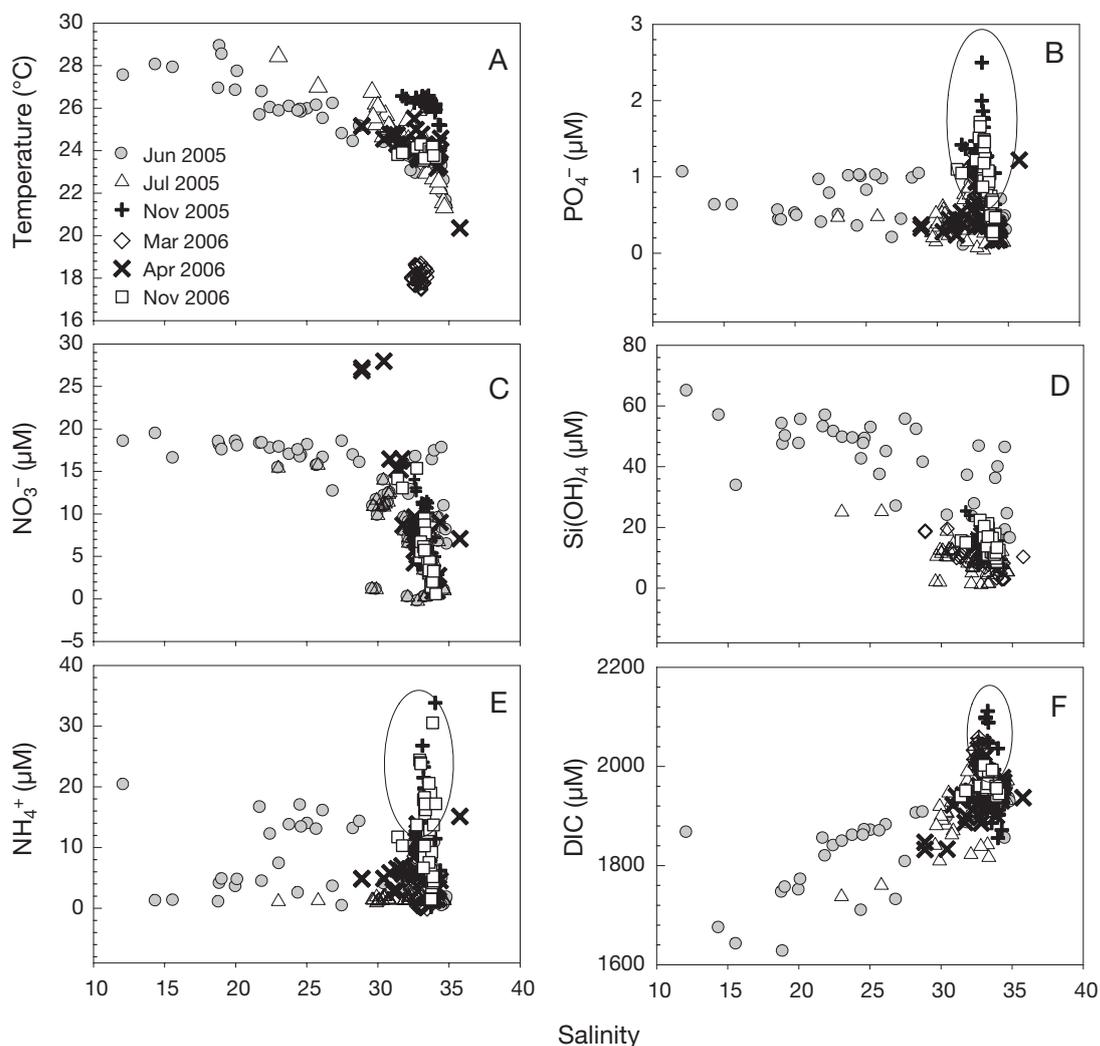
Fig. 2. Temperature, DIC, NO_3^-, $Si(OH)_4$, NH_4^+ and PO_4^- (μM) at the surface and bottom vs. salinity during June, July and November 2005 and March, April and November 2006. Circled data are mainly from Stns 3, 4 and 5 in the dry season (around the sewage discharge outfall)

~26°C (Table 1), and the temperature–salinity diagram showed that warm and low salinity waters were present in the wet season indicating a strong influence of the Pearl River discharge (Fig. 2A). Salinity in the PRE was the lowest (~14) and increased towards eastern waters (~31) (Table 1). Compared to the dry season, salinities decreased by ~30, 20 and 10% near the PRE, VH and eastern waters, respectively, in the wet season (Table 1).

Nutrients

The distribution of NO_3 and $Si(OH)_4$ followed the T-S relationship (Fig. 2C,D), with concentrations decreas-

ing with salinity. In the wet season, NO_3 and $Si(OH)_4$ were high near the PRE due to the freshwater influence from the PRE, whereas they decreased markedly in eastern waters (Fig. 2C,D). The regression lines of NO_3 and $Si(OH)_4$ vs. salinity were often concave upward, which was most likely due to the external additions of NO_3 and $Si(OH)_4$ from sewage effluent.

In contrast to NO_3 and $Si(OH)_4$, NH_4 and PO_4 had different spatial distribution patterns and their concentrations were not significantly correlated with salinity (r < 0.1, p > 0.05, n = 200) (Fig. 2). NH_4 and PO_4 concentrations were relatively high at a salinity of 31 in VH near the sewage discharge site compared with other stations (see the data points within the circles in Fig. 2B,E).
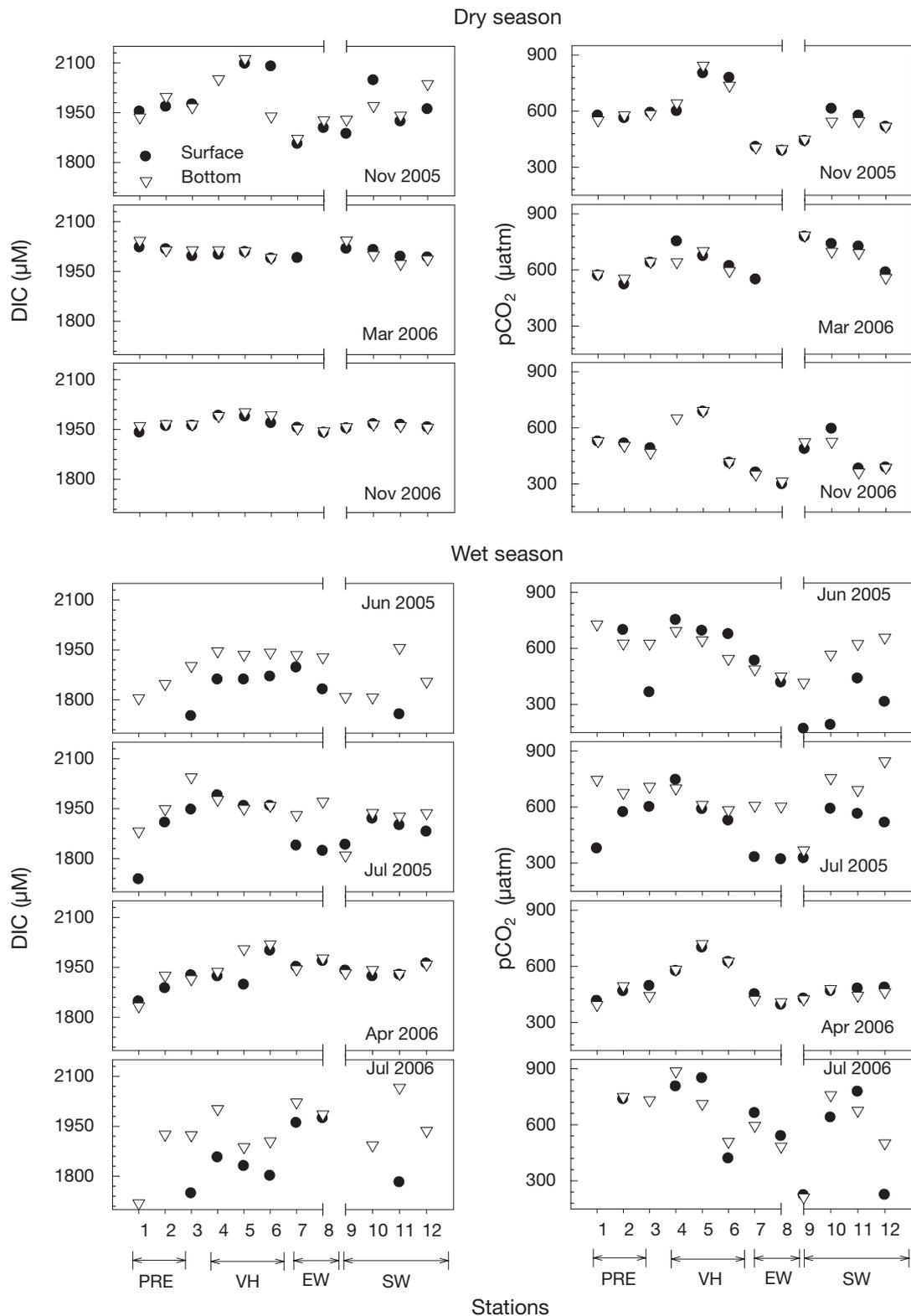
Fig. 3. Spatial distribution of dissolved inorganic carbon (DIC) and partial pressure of CO_2 (pCO_2) at 12 stations during June, July and November 2005 and March, April, July and November 2006. Stations were grouped into 4 sections: PRE (Pearl River estuary), VH (Victoria Harbor), EW (eastern waters) and SW (southern waters). Stn 3 is a transitional station between the PRE and VH. The separation in the *x*-axis between Stns 8 and 9 indicates the separation between the west to east transect and the north to south transect
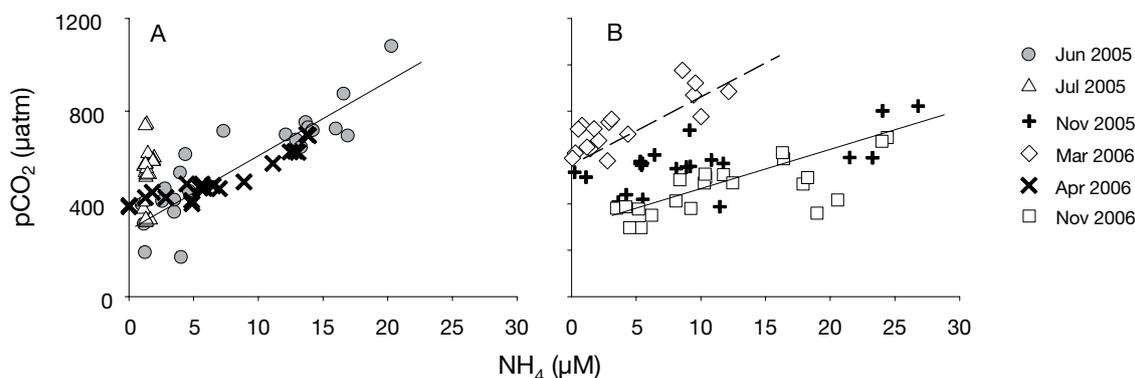
Fig. 4. Linear regression between surface ammonium (NH_4) and temperature-normalized pCO_2 at all stations: (A) in the wet season ($r = 0.8$, $p < 0.05$, $n = 60$), and (B) the dry season (November 2005 and 2006: $r = 0.8$, $p < 0.05$, $n = 40$; March 2006: $r = 0.8$, $p < 0.05$, $n = 20$)

DIC and pCO_2

Overall, DIC increased with salinity in the study area. In the dry season, spatial variations in DIC were not obvious at all 12 stations except in Nov 2005, when DIC increased from ~1850 µM in eastern waters to 2100 µM in VH (Fig. 3). DIC concentrations were ~1950 µM and exhibited no significant difference between the surface and bottom (Fig. 3). In the wet season, DIC concentrations were usually <1950 µM, being lower than the dry season. DIC was usually the lowest at Stn 1 (<1900 µM) and increased towards Stn 8 (~1950 µM) (Fig. 3). In southern waters, DIC was very variable, ranging from ~1500 to 2000 µM (Fig. 3).

In the wet season, pCO_2 levels were consistently low in eastern waters (~400 µatm), but very variable near the PRE (~300 to 1200 µatm) (Fig. 3). With a positive correlation with NH_4 (Fig. 4), pCO_2 was relatively high in VH (>600 µatm) where NH_4 concentrations were also high, as compared to eastern waters (~300 to 600 µatm) (Fig. 3). The lowest pCO_2 (<300 µatm) among all 12 stations occurred at Stn 9 in June 2005 and July 2006 (Fig. 3).

DO and respiration

In the dry season, DO concentrations were ~210 µM near the PRE and eastern waters and ~170 µM in VH (Table 1). In contrast, DO increased in eastern waters (~170 µM) but decreased near the PRE in the summer wet season (Table 1). The lowest DO often occurred in VH year round (Table 1).

DO saturation was usually ~85% in the wet season and increased to ~93% in the dry season near the PRE, while DO saturation was ~80% near VH year round (Table 1), indicating that DO was generally undersaturated. In eastern waters, DO was usually super-

saturated (>100%) in the wet season, but slightly decreased in the dry season (Table 1).

DCR was ~12 to 25 µM d^{-1} and 3 to 13 µM d^{-1} in the wet and dry season, respectively (Table 2), and the bacterial fraction of DCR was ~50 to 80% at Stns 7 and 12 in July and November 2005. Near VH, DCR and BR were ~13 and 11 µM d^{-1}, respectively (Table 2), and hence the bacterial fraction of DCR was >90% year round.

Chl *a*, BA, BP and BGE

In the dry season, surface chl *a* concentrations were constantly <3 mg m^{-3} in eastern waters and VH, but were occasionally high at ~10 mg m^{-3} near the PRE (Fig. 5). In comparison with the dry season, surface chl *a* concentrations did not significantly increase near the PRE in the wet season. However, chl *a* concentrations in eastern waters and VH were generally high in the wet season (~3 to 20 mg m^{-3}) and low in the dry season (~0.1 to 4 mg m^{-3}). The highest surface chl *a* occurred in the southern waters, which was as high as 30 mg m^{-3} (Fig. 5) and corresponded to the lowest pCO_2 (<300 µatm) (Fig. 3).

Near the PRE, BP was negatively correlated with salinity (Fig. 6A). In eastern waters, BP was significantly correlated to chl *a* ($r = 0.2$, $p < 0.05$, $n = 20$) (Fig. 6B). The variations in BA were generally similar to BP (Fig. 5). BP was ~1 to 8 µM C d^{-1} in VH (Stn 5) and ~1 to 3 µM C d^{-1} in eastern waters year round.

In the wet season, bacterial growth efficiency (BGE) exhibited a pronounced gradient with a low value of 0.08 in eastern waters and high values of 0.2 and 0.3 in VH and southern waters, respectively (Table 2). In contrast to winter, there was no gradient, and BGE was ~0.13 in both the eastern waters and near the sewage discharge outfall in VH, due to the invasion of coastal

Table 2. Average (±SD) surface dark community respiration (DCR), bacterial respiration (BR), bacterial fraction of DCR (BF), bacterial growth efficiency (BGE), and empirical BGE calculated with a conversion factor (CF) of 3 kg C mol leucine^{-1} (BGEe). Note: data of DCR were from all 7 cruises with 12 stations; BF, BR, BGE and BGE$_e$ were from cruises in July 2005 and November 2005. –: no measurement; n = number of replicates × number of cruises

Stn	Wet season					Dry season				
	DCR (μM C d^{-1}) (n = 2 × 7)	BR (μM C d^{-1}) (n = 4 × 2)	BF (n = 4 × 2)	BGE (n = 3 × 2)	BGE$_e$ (n = 3 × 2)	DCR (μM C d^{-1}) (n = 2 × 7)	BR (μM C d^{-1}) (n = 4 × 2)	BF (n = 4 × 2)	BGE (n = 3 × 2)	BGE$_e$ (n= 3 × 2)
1	19 ± 9	–		–	–	9 ± 6	–		–	–
2	16 ± 6	–		–	–	9 ± 6	–		–	–
3	12 ± 2	11 ± 2	0.9	0.20 ± 0.02	0.13 ± 0.02	9 ± 6	–		–	–
4	13 ± 3	–		–	–	13 ± 3	–		–	–
5	13 ± 3	11 ± 4	0.9	0.20 ± 0.06	0.13 ± 0.1	12 ± 6	11 ± 3	0.9	0.13 ± 0.02	0.09 ± 0.01
6	19 ± 9	–		–	–	6 ± 0	–		–	–
7	19 ± 6	13 ± 3	0.7	0.08 ± 0.01	0.03 ± 0.01	3 ± 3	3 ± 1	0.9	0.13 ± 0.03	0.09 ± 0.01
8	19 ± 9	–		–	–	3 ± 2	–		–	–
9	21 ± 9	–		–	–	6 ± 3	–		–	–
10	25 ± 9	–		–	–	6 ± 3	–		–	–
11	16 ± 6	–		–	–	9 ± 4	–		–	–
12	18 ± 6	4 ± 1	0.5	0.3 ± 0.02	0.2 ± 0.02	6 ± 3	–		–	–

waters during the period of the downwelling when the NE monsoon winds occurred.

DISCUSSION

Undersaturated DO and supersaturated CO_2

The variability in DO is a measure of water quality, but it is also indicative of ecosystem metabolism. For example, the percentage oxygen saturation (or apparent oxygen utilization) can be used to infer respiration by planktonic organisms in the ocean by assuming that surface oxygen concentration in a water body of low biological activity is close to saturation with the overlying atmosphere (Ito et al. 2004). The average percentage oxygen saturation in VH near the sewage discharge site steadily declined from 83.4% in 1973 to 61.6% in 1983 due to an increase in nutrient loading from sewage effluent (Yung et al. 1999). However, starting in November 2001, about 70% of the sewage in HK has been receiving chemically enhanced primary treatment (CEPT), in which about 7 t per day of suspended solids are removed. Our results showed that despite the removal of some suspended solids, the average percentage oxygen saturation at the surface in VH (Stn 5) was ~78 ± 8% in the wet season and ~85 ± 4% in the dry season during 2005 and 2006 (Table 1), which was significantly higher than that in 1983 (65 ± 5%) (t = 4.5, df = 25, p < 0.05).

Supersaturated CO_2 can be an indication of high respiration and, hence, heterotrophy. The coastal waters of Hong Kong exhibited supersaturated CO_2 concentrations (pCO$_2$ > 370 µatm) at all 12 stations during 2005 and 2006. The exception was that pCO$_2$ at the surface was low (<200 µatm) (Fig. 3) when surface chl a concentrations were >20 mg m^{-3} at Stns 9 and 10 in June 2005 when an algal bloom occurred. The predominance of CO_2 supersaturation suggested that CO_2 release due to respiration exceeded photosynthetic carbon uptake in Hong Kong waters. Previous studies also suggested that estuaries and adjacent waters which receive large external inputs of organic matter may respire more carbon than they produce internally through phytoplankton production (Raymond et al. 2000).

Undersaturated DO and supersaturated CO_2 were also found in some European and U.S. estuaries such as Scheldt and Satilla, with pCO$_2$ > 5000 µatm and DO < 100 µM indicating high respiration due to loadings of organic matter from pollution (Frankignoulle et al. 1998, Gazeau et al. 2004, 2005), or from intertidal marshes (Cai & Wang 1998). In Hong Kong coastal waters, surface pCO$_2$ was usually <1000 µatm, which was lower than upstream in the PRE (>4000 µatm) during the wet season. However, DOC (~170 to 180 µM) in VH (data not shown) was often higher than upstream in the PRE (100 to 170 µM) in the wet season (Zhang et al. 1999). The high DOC and relatively low pCO$_2$ in VH, as compared to upstream in the PRE, indicated that there might not be sufficient time for the bacteria to respire and consume DOC. Strong mixing appears to play an important role in regulating DO and pCO$_2$ in Hong Kong coastal waters. It would take at least 15 d to respire all DO, if DCR is ~13 µM d^{-1} in the wet season, but the average residence time is only ~1.5 to 2.5 d in the wet season in VH (Kuang & Lee 2004).

Influence of the PRE

The eastern waters exhibited the least variations in salinity, DO and DIC between the wet and dry seasons, due to less influence from freshwater discharge. However, near the PRE in the western region, the freshwater discharge from the PRE caused a considerable decrease in DIC and an increase in DOC concentrations in the wet
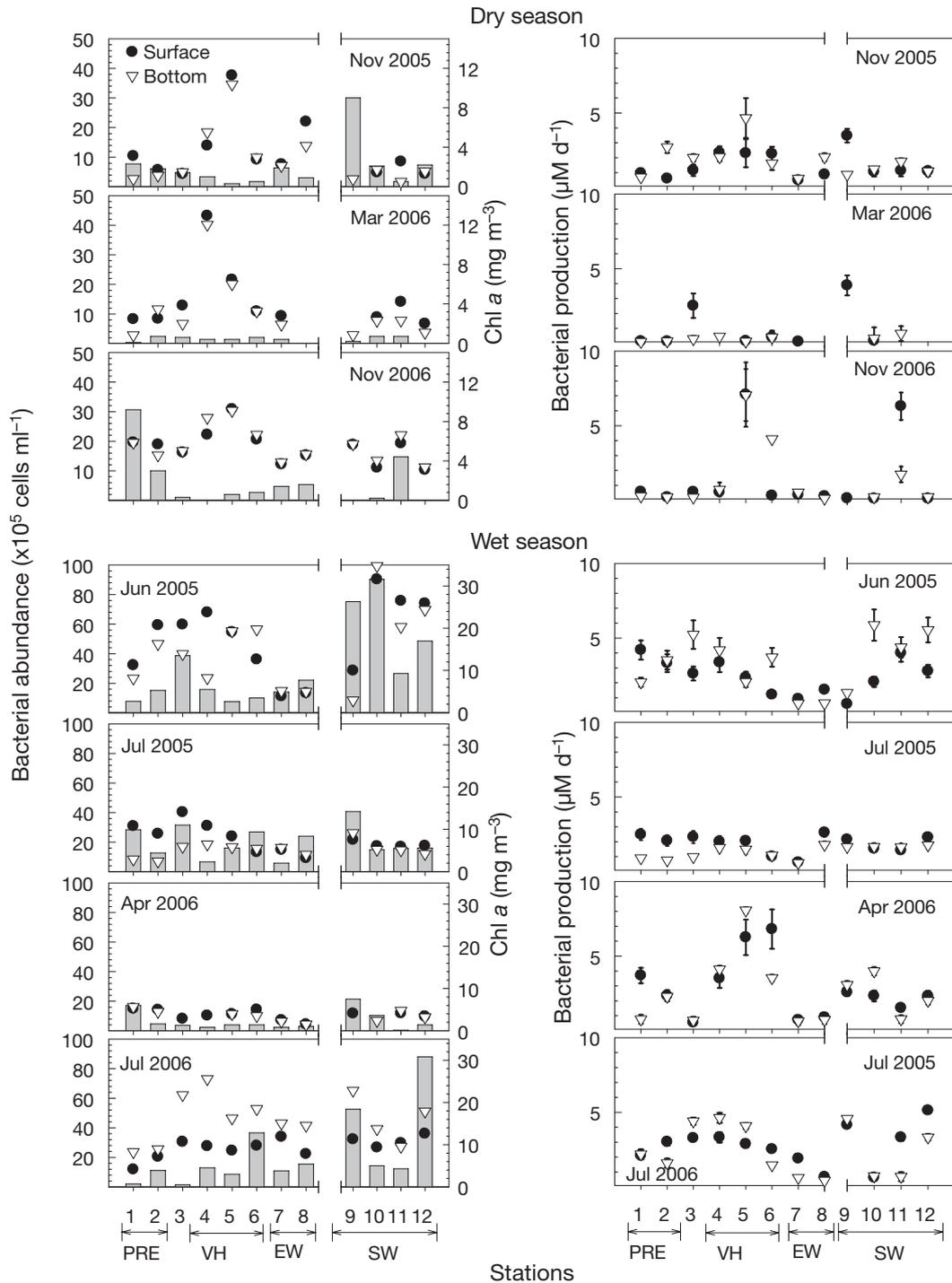

Fig. 5. Bacterial abundance (BA) and bacterial production (BP) along 12 stations during June, July and November 2005 and March, April, July and November 2006 (for abbreviations and explanation see Fig. 3). Stn 3 is a transitional station between the PRE and VH. Surface chl *a* concentrations are also shown as vertical gray bars. Error bars are ±1 SD (for BP only). Note the different scale for the dry and wet seasons
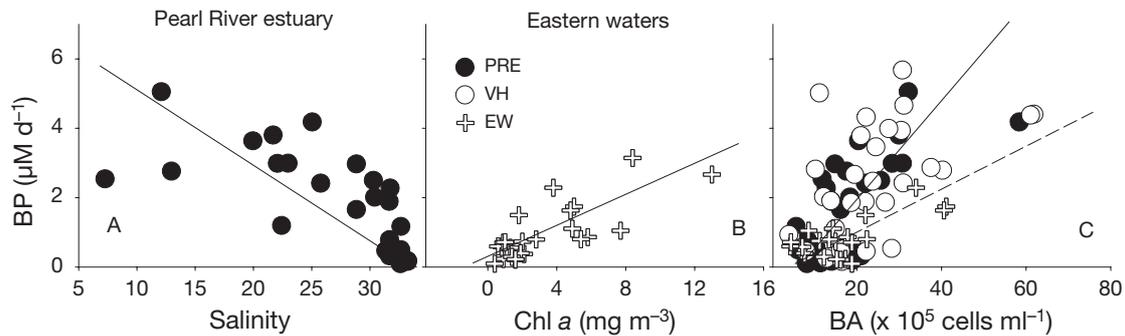
Fig. 6. The relationship of bacterial production (BP) with (A) salinity in the PRE (Pearl River estuary; Stns 1 and 2) ($r = -0.7$, $p <$ 0.05, $n = 20$), (B) chl a in eastern waters (EW) (Stns 7 and 8) ($r = 0.8$, $p < 0.05$, $n = 20$), and (C) bacterial abundance (BA) in the PRE ($r = 0.5$, $p < 0.05$, $n = 20$), VH (Victoria Harbor; $r = 0.5$, $p < 0.05$, $n = 20$) and EW ($r = 0.8$, $p < 0.05$, $n = 19$)

season. NO_3 and $Si(OH)_4$ concentrations were also strongly influenced by the freshwater outflow from the PRE, as indicated by significant correlations with salinity (Fig. 2C,D).

In addition to nutrients, the freshwater from the PRE also appeared to regulate the variations in BP near the PRE, as BP near the PRE was significantly correlated with salinity (Fig. 6A). In contrast, BP in eastern waters was significantly correlated with chl a (Fig. 6B) but not salinity, indicating phytoplankton was an important regulating factor of BP in eastern waters. Due to the dominant influence of high sewage effluent throughout the year in VH, BP exhibited no correlation either with salinity or chl a. In addition, the slope of the regression between BP and BA was higher near the PRE and VH than eastern waters (Fig. 6C), indicating higher BP:BA ratios or higher production per cell near the PRE and VH, likely due to high inputs of nutrients and organic matter.

An important question concerning estuarine ecosystem functionality is whether it is autotrophic or heterotrophic. Recent observations have shown that estuarine systems may release a significant amount of CO_2 into the atmosphere in addition to the export of inorganic/organic matter to marine systems. For example, Frankignoulle et al. (1998) estimated that the amount of CO_2 outgassed from European estuaries is equivalent to 5 to 10% of the present anthropogenic CO_2 emission from Western Europe, and hence estuaries are significant sources of CO_2 to the atmosphere that is higher than the CO_2 emission to the atmosphere from rivers, streams and lakes (Borges et al. 2006). In the Changjiang (Yangtze) River estuary, pCO_2 of 800 to 2000 µatm was observed in mixing zones (Zhang et al. 1999). In the inner PRE, pCO_2 was as high as ~4000 µatm (Zhai et al. 2005). All of these studies demonstrated that anthropogenic organic matter in an estuary can enhance the respiration rate and hence CO_2 production.

Impacts of sewage effluent

As discussed in the last section, higher bottom DO during our sampling periods than in 1983 in VH (Yung et al. 1999) indicates that the water quality has improved. Previous studies documented that sewage effluent was a major source of NH_4 in VH, since NH_4 was higher than on either side of the harbor (Yin & Harrison 2007). Similarly, there was lower DO and higher pCO_2 and BP near the new sewage discharge site than eastern waters and the PRE in most of the sampling months. Hence, the significant correlation between NH_4 and pCO_2 indicated that pCO_2 was strongly influenced by sewage effluent during 2005 and 2006 (Fig. 4), as the organic inputs from sewage effluent supported the production and release of high amounts of CO_2. In addition, sewage effluent might also increase bacterial growth rate, and this suggestion was supported by the test for homogeneity of slopes, which indicated that the slope of BP vs. BA in VH was significantly different from eastern waters ($F_{1, 29} = 6.9$, $p < 0.05$) (Fig. 6).

The range of BP in Hong Kong waters was similar to the Mississippi River, where hypoxia typically occurs during the summer (Rabalais et al. 1994, Rabouille et al. 2008). However, not all estuaries receiving high nutrient loading exhibit high heterotrophy that could lead to hypoxia/anoxia, due to short residence times, or rapid flushing of the bottom layer, as occurred in the James River, a tributary of Chesapeake Bay (Kuo & Neilson 1987). High flushing also reduces hypoxic events in Hong Kong waters.

Significance of BR and BGE

There may be some methodological concerns about the measurement of BR, which are mainly attributed to the filtration process which results in less predators,

broken cells, autotrophic picophytoplankton (<1 μm) passing through the filter and attached bacteria retained on the filter (Pomeroy et al. 1994, Preen & Kirchman 2004). In the present study, larger and attached bacteria were responsible for only a small portion of respiration, since we checked to see if larger and attached bacteria had been removed by 1 μm filtration and found that there was no significant difference in BA between in filtered and nonfiltered waters ($t = 1.5$, df = 5 , p > 0.05). In addition, autotrophic picophytoplankton, which emit red light under green excitation and are distinct from heterotrophic bacteria, were seldom observed in the filtrate under epifluorescence microscopy.

BR is crucial for the estimation of BGE, which is a key factor in understanding bacterial influence on carbon flow in aquatic ecosystems. However, there are only a few studies on BGE in which BR and BP has been measured simultaneously. In those studies, there were always some uncertainties in BGE measurements due to the CFs for BP (Alonso-Sáez et al. 2007). In the present study, CFs (~4.5 ± 3 and ~3.5 ± 1.5 kg C mol leucine^{-1}) were slightly higher than the CF of ~3 kg C mol leucine^{-1} estimated by Pedrós-Alió et al. (1999) in coastal waters, and the theoretical CF of ~3 kg C mol leucine^{-1} by Simon & Azam (1989). In the present study, BGE was 30 to 50 % lower when a different CF was used (Table 2).

BGE varied from ~0.08 to 0.30 in Hong Kong waters and even lower when applying an empirical CF (~3 kg C mol leucine^{-1}), which was in the low range in many other studies (e.g. Biddanda et al. 1994, Reinthaler & Herndl 2005, Alonso-Sáez et al. 2007). The relatively low BGE indicated that most of carbon passing through the bacterial community was largely respired rather than converted into bacterial biomass. Previous studies showed that BGE might be affected by substrate availability and composition in marine systems (Pomeroy & Wiebe 2001). In addition, it has been suggested that BGE is high where carbon limitation and no inorganic nutrient limitation are found (Alonso-Sáez et al. 2007). Nutrient limitation might be one of explanations for the relatively low BGE in Hong Kong waters, since phosphorus addition could enhance BGE in Hong Kong waters (X. Yuan et al. unpubl. data). In addition, the variability in BGE in Hong Kong waters was mainly due to the variations in BP, as BR varied from 1- to 3-fold over a seasonal cycle (Table 2), while BP varied up to >10-fold (Fig. 5), in agreement with a previous study (Reinthaler & Herndl 2005).

The bacterial fraction of the DCR was higher at Stn 5 (~92 ± 6 %) than Stns 7 and 12 (80 ± 15 %) (Fig. 7A), suggesting that larger plankton was less

important near the sewage discharge outfall in terms of respiration. The bacterial fraction of the DCR (~70 to 90 %) was higher than previous estimations (12 to 59 %) (Robinson & Williams 2005) but similar to that in the coastal Mediterranean (89 %) (Vázquez-Domínguez et al. 2007) and in the southern Bay of Biscay (70 %) (González et al. 2003). BR was a dominant contributor to oxygen consumption in Hong Kong waters, which supports the notion that heterotrophy in Hong Kong waters was mainly due to BR. Such high BR would certainly consume a high amount of organic carbon discharged from the PRE and sewage effluent as well as autochthonous labile DOC (e.g. phytoplankton exudation).

Apart from oxygen consumption, the bacterial contribution to the air-sea efflux of gaseous CO_2 was also considerable (Fig. 7C). BR accounted for a gaseous CO_2 efflux of ~3 to 10 mmol C m^{-2} d^{-1} (Fig. 7C,D), which was only ~50 % (varying from 16 to 130 %) of the total CO_2 efflux, suggesting that there should be another source for CO_2 release, since the utilization of CO_2 by phytoplankton must offset some portion of the CO_2 efflux. The contribution of BR to the CO_2 efflux was ~20 to 100 % of the CO_2 efflux in the York River estuary based on a different estimation method (Ray-

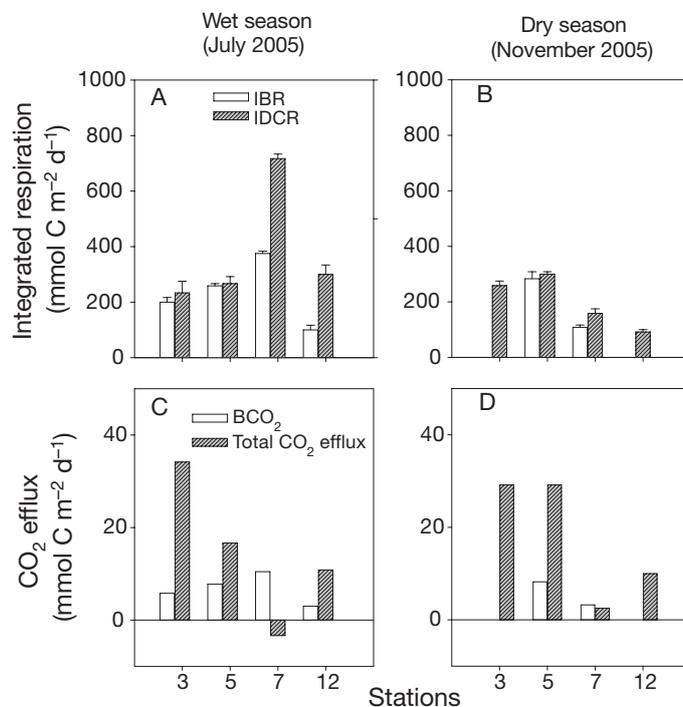
Fig. 7. Vertically integrated bacterial respiration (IBR) (n = 4) (see integrated depths in Table 1); integrated dark community respiration (IDCR; n = 2) at Stns 3, 5, 7 and 12 in (A) July 2005 and (B) November 2005. Contributions of BR to CO_2 efflux (BCO$_2$) and total CO_2 efflux in (C) July 2005 and (D) November 2005. Bacterial respiration was not measured at Stns 3 and 12 in November 2005. Error bars are ±1 SD

mond & Bauer 2000). As Raymond & Bauer (2000) suggested, the large discrepancy between contributions of BR to CO_2 efflux and total CO_2 efflux must arise from (1) water-column remineralization of organic carbon in the >1 µm size fraction, and (2) sediment respiration. In addition, CO_2 efflux might be increased by rivers inputs, since rivers are generally highly supersaturated in dissolved CO_2 (Frankignoulle et al. 1998, Raymond et al. 2000). Coastal upwelling may also affect the carbon balance and pCO_2 by bringing higher DIC from greater depths into nearshore waters (Huthnance 1995, Wollast 1998). The influence of such physical processes on CO_2 flux might also be important in Hong Kong waters when upwelling occurs due to the southwest monsoon in summer and downwelling induced by the northeast monsoon in winter.

Our results demonstrated that Hong Kong waters were net heterotrophic, with a net efflux of CO_2 from surface waters, which suggested that DCR exceeded gross primary production. Kemp et al. (1997) and Hopkinson & Vallino (1995) concluded that the metabolic balance between respiration and primary production in estuaries and the nearby coastal zone depended on the ratio of inputs of dissolved inorganic nutrient to labile organic carbon. With increased eutrophication, net ecosystem metabolism could shift and become more heterotrophic or autotrophic depending on the ratio (Ducklow & McCallister 2004). In Hong Kong waters in the summer, there might be an excess of labile organic carbon relative to dissolved inorganic nutrients, especially P concentrations in the summer, as a P addition increased BGE when inorganic nutrients were relatively deficient (X. Yuan et al. unpubl. data).

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